AUG 3 1917

THE JOURNAL

OF

EXPERIMENTAL MEDICINE

EDITED BY

SIMON FLEXNER, M.D.

VOLUME XXVI, NO. 2 AUGUST 1, 1917

PUBLISHED MONTHLY

AT 2419-21 GREENMOUNT AVE., BALTIMORE, MD.

BY THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

Entered as second-class matter at the Post Office at Baltimore, Md., under the Act of March 3, 1879

COPYRIGHT, 1917, BY THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

WAVERLY PRESS
THE WILLIAMS & WILKINS COMPANY
BALTIMORE, U. S. A.

ERRATUM.

On page 119, Vol. XXVI, No. 1, July 1, 1917, line 1, read *pathology* for *pathogenicity*.

STUDIES IN GLOMERULONEPHRITIS.

I. A Quantitative Study of the Reaction of the Kidney to Diphtheria Toxin.

By HAROLD KNIEST FABER, M.D.

(From the Laboratory of Pediatrics of Stanford University Medical School, San Francisco.)

Plates 9 and 10.

(Received for publication, March 16, 1917.)

In investigating the pathogenesis of acute diffuse glomerulonephritis, it was thought that it might be of value to test the hypothesis that a soluble poison circulating in an active state in the blood will exert a more intense action in the glomerulus than in other tissues, because at this point water is lost from the blood, and any poison present, provided it was not excreted with the water, would necessarily become concentrated and its powers of injury intensified. Such a condition of increased concentration may indeed be assumed *a priori* to occur, the question being whether it is great enough to produce recognizably heightened damage.

For the purpose of testing this hypothesis, diphtheria toxin was selected as answering the experimental requirements. Injected into the blood of non-immune animals it is actively and directly toxic and it does not pass through the glomerular filter into the urine.

Our problem, then, is to compare the effects of different dosages of this poison, and to determine whether small amounts of toxin, injected intravenously, will produce glomerular lesions before demonstrable injury occurs elsewhere, and whether larger amounts produce damage proportionally greater in the tufts than in other parts of the renal tissue. With full dosages, of course, the well recognized picture of a pannephritis (or pannephrosis) is to be expected.

The studies of Oertel and of Councilman, Mallory, and Pearce are perhaps the most important ones treating of the disease in man, and the papers of Wade, who first described nephritis in diphtheria, Wagner, Brault, Fürbringer, Fischl,

Schrakamp, von Kahlden, Bernhard and Felsenthal, Reiche, and Katzenstein may also be mentioned. These studies make it clear that both tubular degeneration and glomerular alterations occur in the course of human diphtheria. The glomerular lesions consist of swelling of the endothelium, dilatation of the loops, various degrees of karyorrhexis of the endothelial nuclei and, in more advanced cases, hyalinization and obliteration of the tuft occur.

Experimental investigations have been made by Roux and Yersin, Spronck, Babes, von Kahlden, Enriquez and Hallion, Baldassari, Welch and Flexner, Flexner, Schlayer and Hedinger, Takayasu, Lyon, Karsner and Denis, Pearce and Eisenbrey, Frothingham, and Bailey. These investigators have presented anatomical or functional evidence of glomerular as well as of tubular injury in experimental diphtheria or diphtherial intoxication. It is clear from a consideration of the work of Baldassari and especially of Flexner that diphtheria toxin is a general cellular poison without specific chemical affinity for any one type of tissue.

Charrin and Moussu showed that diphtheria toxin does not diffuse through animal membranes; Rodet and Guéchoff, that it does not diffuse through collodion;[1] Dzerzhgovski and Onufrovich, that it does not diffuse through the renal tissues when the excised kidney is perfused with a 10 per cent solution in blood; and Bomstein, that it is not excreted into the urine.

Diphtheria toxin, therefore, appears to be well suited to the purposes of the present experiments.

EXPERIMENTAL.

The toxin[2] employed had a strength of 200 minimum lethal doses per cc. when tested April 18, 1916. No evidence of deterioration was noted during the present series of experiments. All injections were made intravenously in quantities of 1 cc., made up in 0.85 per cent salt solution. The rabbits were in most cases young, weighing between 1,000 and 1,500 gm., and were, in so far as external signs showed, in good health. The animals that did not die spontaneously were killed by a blow on the neck and immediately autopsied. The tissues were fixed in Orth's solution with a 5 per cent addition of glacial acetic acid, and embedded in paraffin. Sections were stained by Van Gieson's method, with hematoxylin and eosin, and in a few

[1] Roux and Yersin found some diffusion through parchment paper. The pinholes which can be readily seen in most samples of parchment paper with a hand lens probably explain this discrepancy.

[2] The toxin was prepared at the Cutter Laboratory of Berkeley, California, for use in the Schick test.

instances by Weigert's method for fibrin. Frozen sections were not made. The results are shown in the following table and protocols.

TABLE I.

Rabbit No.	Date of injection.	Weight of rabbit.	Toxin injected.	Dosage per kilo of body weight.	Length of life after injection.	Killed or died.
	1916	*gm.*	*cc.*	*cc.*	*days*	
1	Sept. 6	1,475	0.001	0.00068	3	K.
2	" 6	1,475	0.001	0.00068	3	"
3	" 6	1,475	0.001	0.00068	3	"
4	" 6	1,420	0.001	0.0007	3	"
5	May 2	1,000	0.001	0.001	3	D.
6	Sept. 6	1,500	0.002	0.0013	3	K.
7	" 6	1,450	0.002	0.0014	3	"
8	" 6	1,400	0.002	0.0014	3	"
9	" 6	1,340	0.002	0.0015	3	"
10	" 6	1,340	0.003	0.0022	3	"
11	" 6	1,360	0.003	0.0022	3	"
12	May 2	1,300	0.003	0.0023	10	"
13	Sept. 6	1,280	0.003	0.0023	3	"
14	" 6	1,225	0.003	0.0024	3	"
15	Apr. 27	1,250	0.005	0.004	1½	D.
16	Sept. 1	1,530	0.010	0.0065	2	"
17	" 1	1,530	0.010	0.0065	2	"
18	" 1	1,500	0.010	0.0067	2	"
19	" 1	1,500	0.010	0.0067	2	"
20	" 1	1,450	0.010	0.0069	½	"
21	" 1	1,330	0.010	0.0075	2	"
22	" 1	1,420	0.020	0.0141	2	"
23	" 1	1,300	0.020	0.0154	1	"

PROTOCOLS.

Rabbit 1.—Dosage 0.00068 cc. of toxin per kilo. Combined weight of kidneys 11.9 gm. The macroscopic and microscopic appearance is normal.

Rabbit 2.—Dosage 0.00068 cc. per kilo. Combined weight of kidneys 13.7 gm. Macroscopically the cortex and outer part of the medulla are slightly congested. Microscopically normal except for a slight amount of granular exudate in the capsular space.

Rabbit 3.—Dosage 0.00068 cc. per kilo. Combined weight of kidneys 12.3 gm. Macroscopically normal. Microscopically the glomeruli are somewhat larger than normal; the endothelial cells are slightly increased in number, and the nuclei of the endothelium both in the tufts and the intertubular capillaries are occasionally pyknotic.

Rabbit 4.—Dosage 0.0007 cc. per kilo. Combined weight of kidneys 16 gm. Macroscopically the boundary zone of the medulla is congested. Microscopically the endothelial cells of the tufts are somewhat increased in number.

Rabbit 5.—Dosage 0.001 cc. per kilo. Combined weight of kidneys 9.5 gm. Macroscopically some congestion of boundary zone. Microscopically some increase of the endothelial cells of the tufts, the nuclei of which are occasionally pyknotic. Occasional pyknotic nuclei also in the tubular epithelium. A distinct increase of leukocytes in the tufts.

Rabbit 6.—Dosage 0.0013 cc. per kilo. Macroscopically the boundary zone of the medulla is congested. Microscopically the glomeruli are in part enlarged and most of them are greatly distended with blood. The endothelial nuclei are frequently pyknotic, and there is a considerable increase in the number of endothelial cells in the tufts. The leukocytes in the tufts are somewhat increased in number.

Rabbit 7.—Dosage 0.0014 cc. per kilo. Combined weight of kidneys 13.5 gm. Macroscopically moderate congestion of the medulla. Microscopically the glomeruli show swelling of the endothelial cytoplasm, some loops are distended and empty, many of the nuclei are pyknotic, and there is an increase in the number of endothelial cells (Figs. 1 and 2).

Rabbit 8.—Dosage 0.0014 cc. per kilo. Combined weight of kidneys 16 gm. Macroscopically normal. Microscopically some of the glomeruli are much enlarged and the endothelium generally is much swollen, with many empty loops; the nuclei are moderately increased in number and many are pyknotic or misshapen. There are some desquamated epithelial cells in the capsular space and in the tubules there are a good many hyaline casts.

Rabbit 9.—Dosage 0.0015 cc. per kilo. Combined weight of kidneys 10.8 gm. Macroscopically normal. Microscopically marked increase in the number of endothelial nuclei of tufts, a considerable number of which are pyknotic. Moderate endothelial swelling. Occasional glomeruli are much enlarged.

Rabbit 10.—Dosage 0.0022 cc. per kilo. Combined weight of kidneys 15.5 gm. Macroscopically moderate general congestion. Microscopically marked swelling of the glomerular endothelium with dilatation of the loops; many pyknotic nuclei, and slight increase in the number of cells. A considerable number of hyaline casts. The epithelium of the tubules is granular in appearance, but there is no desquamation and no pyknosis of the nuclei.

Rabbit 11.—Dosage 0.0022 cc. per kilo. Combined weight of kidneys 15 gm. Macroscopically nothing abnormal. Microscopically marked increase in the number of the endothelial cells of the tufts, of which the cytoplasm is swollen and the nuclei are pyknotic. Some dilatation of the loops. In places, some stratification of the parietal epithelium of the capsule of Bowman is seen. The tubular epithelium contains pyknotic nuclei in places; the cytoplasm is granular and shows beginning desquamation. Some hyaline casts.

Rabbit 12.—Dosage 0.0023 cc. per kilo. Combined weight of kidneys 12 gm. Macroscopically moderate general congestion. Microscopically swelling and

numerical increase of the endothelial cells of tufts, with occasional pyknotic nuclei. Changes are not so marked as in preceding.

Rabbit 13.—Dosage 0.0023 cc. per kilo. Combined weight of kidneys 9.6 gm. Macroscopically general pallor. Microscopically glomeruli large with dilated loops and swollen endothelium, the cells being somewhat increased in number with occasional pyknotic nuclei. The tubular epithelium shows moderate cloudy swelling.

Rabbit 14.—Dosage 0.0024 cc. per kilo. Combined weight of kidneys 13.8 gm. Macroscopically moderate general congestion. Microscopically the glomeruli are very large with many dilated empty loops (Fig. 3). The endothelium is greatly swollen. The capsular space is almost obliterated. There are a few small intraglomerular hemorrhages. Considerable desquamation of the tubular epithelium, many of the nuclei of which are pyknotic, misshapen, and in places extruded into the lumen. There are many hyaline casts.

Rabbit 15.—Dosage 0.004 cc. per kilo. Combined weight of kidneys 22 gm. Macroscopically they are much swollen and intensely congested; the cortical surface shows whitish, poorly defined patches, which on the cut surface are seen as radial bands extending into the medulla; probably the medullary rays contrasting with the deeply congested cortical labyrinths. Microscopically the majority of glomeruli are hemorrhagic and converted into cysts of blood which is mainly fresh but in places shows beginning coagulation (Fig. 4). These hemorrhages are mostly confined within the intact epithelium of the visceral layer of Bowman's capsule, but in places the latter has ruptured and flooded the capsular space and the proximal tubules with red blood cells. The glomerular nuclei that are still recognizable are pyknotic or fragmented. In the tubules the epithelium is in places granular and the nuclei are occasionally pyknotic but the evidences of severe damage are much less than in the glomeruli. There is no evidence of repair.

Rabbit 16.—Dosage 0.0065 cc. per kilo. Combined weight of kidneys 26 gm. Macroscopically the kidneys are greatly enlarged and intensely congested with several small superficial hemorrhages under the capsule. Microscopically the appearance is practically that of Rabbit 15, except that more of the hemorrhages are coagulated, showing masses of fibrin, and the epithelial degeneration in the tubules is more advanced (Figs. 5 and 6).

Rabbits 17 and 18.—Practically the same picture as No. 15.

Rabbit 19.—Dosage 0.0067 cc. per kilo. Combined weight of kidneys 20 gm. Macroscopically the kidneys are dark purple in color, with several subcapsular hemorrhages; the cut surface shows a much thickened cortex in which there are many small hemorrhages, and the glomeruli are seen as fine red points. The medullary rays are paler than the labyrinths. Microscopically the hemorrhages are seen to be from the intertubular capillaries both in the cortex and the outer medullary zone. In the tufts, the glomerular endothelium has undergone acute degeneration, the lumina being filled with an amorphous granular material, and the nuclei are all pyknotic or fragmented except the large epithelial nuclei, which

in most places appear to be little damaged. There is marked coagulation necrosis of the tubular epithelium, especially of the proximal tubules, many of which show extruded pyknotic nuclei and desquamated epithelium in the lumen (Fig. 7).

Rabbit 20.—(Died 12 hours after injection.) Dosage 0.0069 cc. per kilo. Combined weight of kidneys 19 gm. Macroscopically they are swollen and dark purple in color without gross hemorrhages. Microscopically there is extreme swelling of the glomerular endothelium and the loops are dilated and empty. The nuclei are pyknotic. The epithelium of the tubules shows coagulation necrosis with the nuclei pyknotic. The intertubular capillaries are greatly dilated.

Rabbit 21.—Dosage 0.0075 cc. per kilo. Combined weight of kidneys 19 gm. Macroscopically the kidneys are deep purple with congestion, but there are no gross hemorrhages. Microscopically the glomerular endothelium is in places swollen, but as a rule it is definitely necrotic and disintegrated, the lumina being filled with finely granular debris. The nuclei are pyknotic and fragmented. There are occasional intraglomerular hemorrhages. The epithelial degeneration of the tubules is marked, but no formed casts are present.

Rabbit 22.—Dosage 0.0141 cc. per kilo. Combined weight of kidneys 16 gm. Macroscopically intense congestion. Microscopically there is disintegration of the capillary endothelium in the tufts and in the intertubular capillaries with thrombi of finely granular material. There are many greatly dilated empty loops and a large amount of serous exudate (Fig. 8) containing in places red blood cells. Marked epithelial degeneration in the tubules.

Rabbit 23.—Dosage 0.0154 cc. per kilo. Combined weight of kidneys 15 gm. Macroscopically intense congestion. Microscopically extreme swelling of the glomerular endothelium with beginning necrosis, and thrombosis of the loops. Many of the loops are dilated and empty. The epithelial degeneration is not advanced, but the cytoplasm appears somewhat granular and occasional pyknotic nuclei are seen. The rabbit died 24 hours after injection.

The changes described in the protocols indicate, in spite of slight differences at the same level of dosage, that the severity of the lesion is roughly proportional to the size of the dose administered. It is evident that the primary change is in the endothelium of the tuft. It is possible that a simultaneous alteration of the intertubular capillaries occurs, but is difficult to detect, because of the intimate relation between them and the walls of the renal lymphatics. Even hemorrhage from these capillaries is hard to recognize, since the escaping blood follows the lines of cleavage which closely correspond to the normal path of the small vessels. However, it was found that the small flat nuclei between the tubules were frequently pyknotic and this occurred even in the rabbits receiving the smallest doses. With

larger doses, the capillaries, especially in the inner cortical and outer medullary zones, were found to be greatly dilated and full of blood.

With dosages approximating 0.0007 cc. of diphtheria toxin per kilo in rabbits very slight changes of any kind are produced in the kidney, though occasional deeply staining endothelial nuclei and a slight increase of these nuclei in the glomeruli give evidence that some damage has been inflicted. With dosages between 0.001 and 0.0015 cc. per kilo, changes of increasing severity are found, consisting of evidences of both nuclear and protoplasmic irritation and injury such as multiplication of the nuclei and swelling of the cytoplasm of the glomerular endothelium. With these doses evidence of epithelial injury in the tubules is present, but not striking. With dosages over 0.002 cc. per kilo the glomerular changes become more severe as shown by marked endothelial swelling, extensive pyknosis of the nuclei, and marked enlargement of the tufts with dilatation of those which remain pervious. At this stage, degeneration of the tubular epithelium is indicated by pyknosis and karyorrhexis, desquamation of the cells, and the formation of numerous casts, but even in the parenchyma the presence of pyknotic nuclei in the intertubular capillaries suggests the possibility of primary vascular damage. In the glomeruli it is sometimes possible to distinguish the epithelial nuclei of the visceral and parietal layers of the capsule apparently unharmed, while in the same tuft the endothelial nuclei show advanced injury. With doses of 0.004 cc. per kilo and more, the glomerular capillaries rupture and globular hemorrhages confined by the epithelial syncytium of the capsule are seen. In some instances the capsule, too, gives way and the capsular space and proximal tubules are found to be flooded with newly extravasated blood cells.

In so far as diphtheria toxin causes glomerular hemorrhages, it is apparently by simple rupture of the capillaries, not by diapedesis. The lesion sometimes observed in human hemorrhagic glomerular nephritis, in which fibrin, red blood cells, and leukocytes exude into the capsular space, is notably absent. At most, the only real exudate into the space is a homogeneous, albuminous material, observed in one kidney of the present series, though desquamated epithelial cells from the capsule may give a superficial resemblance to a true exudate.

With doses of 0.0065 cc. per kilo and more, such hemorrhages are less constant, and instead we find thrombotic masses within the necrotic capillaries. The nature of these masses is somewhat difficult to determine. They do not take the Weigert stain for fibrin, but with Van Gieson's stain they are brownish in color. Under a low power objective they form interlacing and branching figures about the size and conformation of the glomerular capillaries. Closely examined with the high power objective they are seen to consist of very finely granular amorphous material usually completely occluding the capillaries but in some places lying in the center of the lumen. It seems probable that the material is the necrotic and desquamated cytoplasm of the endothelium, a supposition which is strengthened by the presence in the material of faintly staining or pyknotic and often fragmented nuclei. It is possible also that some of this material may be embolic, derived from the degenerated intimal endothelium of the afferents, a process observed by von Kahlden in some cases of human diphtherial nephritis. With these high dosages there is evidence of rapid death of the whole kidney, with pyknotic and fragmented nuclei, and granular, desquamated, or coagulated cytoplasm. Such doses are usually fatal within 48 hours. The maximum effect is obtained apparently with doses of about 0.008 cc. per kilo, the pathological changes resulting from 0.015 cc. per kilo being nearly the same as with the former amount.

Leukocytes within the vessels and occasionally surrounding the vessels or the glomerular capsule were sometimes found but, as the protocols show, this phenomenon bears no relation to the dosage of toxin and should probably be regarded as due to other causes. A possible rôle of the leukocytes in these kidneys is to remove necrotic fragments by phagocytosis. A mobilization of leukocytes as a result of leukotactic action by the toxin is not shown by the present series of experiments.

DISCUSSION.

It is not necessary to assume a specific affinity of diphtheria toxin for endothelium in order to account for the vascular injury found in the above experiments. Babes and later Baldassari determined that the primary action of diphtheria toxin is upon the nuclei of the body

cells in general. As regards the kidney it is possible that the small amount of cytoplasm of the flat endothelial cells acts as a less efficient buffer for the nucleus against the attack of the toxin than the abundant cytoplasm of the tubular epithelium, but a simpler explanation is that the toxin, being brought to the kidney by the blood stream attacks the tissue with which it comes in direct contact; namely, the endothelium of the vessels.

As regards the location of severe injury in the tuft the experimental findings stand in fairly close agreement with the theoretical expectation that the glomerulus should show an especially severe grade of injury because the circulating toxin reaches at this point a relatively high concentration. It was actually found that the earliest recognizable alteration, and with larger doses, the severest lesions were in the glomeruli, and perhaps the intertubular capillaries, and that these lesions were essentially endothelial.

These endothelial glomerular lesions were observed in the present series in all degrees of intensity, progressing through the following stages: (1) cellular proliferation; (2) swelling of the cytoplasm with partial or total occlusion of the capillaries and beginning degeneration of the nuclei; (3) rupture of the capillaries with hemorrhage; (4) necrosis and disintegration of the cytoplasm with blockage of the capillaries with protoplasmic debris.

It has been shown by Charrin and Moussu that diphtheria toxin does not diffuse through animal membranes, and by Rodet and Guéchoff that it does not diffuse through collodion, and we may infer that during life physical dialysis of toxin through the glomerulus and tubules does not occur. To explain the action of diphtheria toxin on the tubular epithelium we have to assume either a selective activity of the tubular cells or an effect secondary to vascular injury. That the first of these assumptions is unlikely is shown by the fact established by Bomstein that toxin is not excreted into the urine, and by the findings of Dzerzhgovski and Onufrovich that toxin is not retained by the kidney in perfusion experiments. Anatomically the secondary nature of the epithelial lesions is indicated by the fact that with small doses of toxin glomerular lesions are recognizable before tubular degeneration appears and that with doses of medium size the glomerular lesions are much severer than the tubular. In short, endothelial injury is predominant.

A point deserving emphasis is the slight anatomical evidence of excretion of toxin from the tufts. This is shown by the small amount of damage to Bowman's capsule and the small quantity of exudate in the capsular space. It was notable in many kidneys that, while the endothelial cells were necrotic and disorganized, the epithelial cells were surprisingly well preserved and the parietal layer of the capsule little altered. A striking confirmation of the truth of this observation was the frequent occurrence of hemorrhages obliterating practically the entire capillary structure of the tuft, but confined within the unruptured epithelial syncytium of the visceral layer of Bowman's capsule. Only with relatively enormous doses of toxin and then in one case only was there a marked exudate in the capsular space such as we find in the so called extracapillary form of glomerulonephritis. To explain the pathogenesis of the latter form of renal disease, we must perhaps adduce other factors which possibly call into action some specific excretory activity of the glomerular epithelium.

The lesions described in the present experiments, at least those obtained with subhemorrhagic doses of toxin, with their evidence of injury within the tuft are strongly reminiscent of those described by Volhard and Fahr under the name of intracapillary glomerulonephritis. They say:

> "The glomeruli are strikingly large. . . . containing little blood, and the cellular elements within the tufts are enormously increased. The loops are swollen and often become adherent and hyalinized. . . . In the capsular space occasional desquamated epithelium, a little coagulated albumin and here and there red blood cells are seen. . . . but usually the space is empty."

We shall not in my opinion, be far from the truth if we apply our observations and deductions to the pathogenesis of the intracapillary form of glomerulonephritis in general, and assume that this form of disease is the result of the concentration, due to loss of water in the tuft, of poisons circulating in an active state in the blood. It seems highly probable that such a process plays at the least a major part in the production of these lesions.

SUMMARY.

1. Diphtheria toxin of known strength, injected intravenously into rabbits in single doses, produces lesions which are first evident in the endothelium of the tufts, and possibly in the intertubular capillaries.

2. The lesions of greatest severity with medium doses are also found in the tufts.

3. Evidence is presented to show that epithelial damage in the tufts and especially in the tubules is secondary to the vascular injury.

4. The severity of the lesions was roughly proportional to the amount of toxin injected. Hemorrhages did not occur with doses of less than 0.0023 cc. of toxin per kilo of body weight and were not extensive with doses of less than 0.004 cc. per kilo.

5. It is suggested that the localization of lesions in the tufts is due to the concentration of toxin in the blood that follows the loss of water at this point.

6. It is further suggested that this inference may be extended to explain the pathogenesis of the so called intracapillary form of glomerulonephritis.

BIBLIOGRAPHY.

Babes, V., Untersuchungen über den Diphtheriebacillus und die experimentelle Diphtherie, *Virchows Arch. path. Anat.*, 1890, cxix, 460.

Bailey, C. H., The production of arteriosclerosis and glomerulonephritis in the rabbit by intravenous injections of diphtheria toxin, *J. Exp. Med.*, 1917, xxv, 109.

Baldassari, L., Ueber die Wirkung der Diphtherietoxine auf den Zellkern, *Centr. allg. Path. u. path. Anat.*, 1896, vii, 625.

Bernhard, L., and Felsenthal, S., Beitrag zur pathologischen Anatomie der diphtherieniere, *Arch. Kinderheilk.*, 1893, xvi, 308.

Bomstein, Z., quoted by Oppenheimer, C., Toxine und Antitoxine, Jena, 1904, 79.

Brault, Note sur les lésions du rein dans l'albuminurie diphthéritique, *J. anat. et physiol.*, 1880, xvi, 673.

Charrin and Moussu, Influence des dialyses ou filtrations intra-organiques sur les principes toxiques, *Compt. rend. Soc. biol.*, 1900, lii, 694.

Councilman, W. T., Mallory, F. B., and Pearce, R. M., A study of the bacteriology and pathology of two hundred and twenty fatal cases of diphtheria, Boston, 1901.

Dzerzhgovski, S. K., and Onufrovich, Experimental researches into the relations of various organs to diphtheritic toxins, *Arch. biol. nauk.*, 1897–98, vi, 40.

Enriquez and Hallion, Rein granuleux expérimental avec hypertrophie du coeur par toxine diphthérique, *Compt. rend. Soc. biol.*, 1894, series 10, i, 776.

Fischl, J., Zur Kenntniss der Nierenaffection bei der Diphtherie, *Z. klin. Med.*, 1884, vii, 427.

Flexner, S., The pathology of toxalbumin intoxication, *Johns Hopkins Hosp. Rep.*, 1897, vi, 259.

Frothingham, C., Jr., A glomerular and arterial lesion produced in rabbits' kidneys by diphtheria toxin, *J. Med. Research*, 1914, xxx, 365.

Fürbringer, P., Zur Klinik und pathologische Anatomie der diphtherischen Nephritis, *Virchows Arch. path. Anat.*, 1883, xci, 385.

von Kahlden, C., Die Aetiologie und Genese der acuten Nephritis, *Beitr. path. Anat. u. allg. Path.*, 1892, xi, 441.

Karsner, H. T., and Denis, W., A further study of nitrogen retention in the blood in experimental acute nephritis, *J. Exp. Med.*, 1914, xix, 259.

Katzenstein, M., Ueber die sekundäre Veränderungen der Organe bei Rachendiphtherie, Munich, 1895.

Lyon, G., Inflammatory changes in the kidney: an experimental study of the action of some toxins and poisons upon the kidney and also upon the spleen, *J. Path. and Bacteriol.*, 1904, ix, 400.

Oertel, M. J., Die Pathogenese der epidemischen Diphtherie nach ihrer histologischen Begründung, Leipsic, 1887.

Pearce, R. M., and Eisenbrey, A. B., A physiological study of experimental nephritis due to bacterial poisons and cytotoxic sera, *J. Exp. Med.*, 1911, xiv, 306.

Reiche, F., Nierenveränderungen bei Diphtherie, *Centr. inn. Med.*, 1895, xvi, 1209.

Rodet, A., and Guéchoff, Sur les propriétés des sacs de collodion et leur rôle en bactériologie, *Compt. rend. Soc. biol.*, 1900, lii, 965.

Roux, E., and Yersin, A., Contribution à l'étude de la diphthérie, *Ann. Inst. Pasteur*, 1888, ii, 629–661; 1889, iii, 273.

Schlayer and Hedinger, Experimentelle Studien über toxische Nephritis, *Deutsch. Arch. klin. Med.*, 1907, xc, 1.

Schrakamp, F., Ueber die Organveränderungen bei der Diphtherie, *Arch. Kinderheilk.*, 1888, ix, 161.

Spronck, C. H. H., Le poison diphthérique considéré principalement au point de vue de son action sur le rein, *France méd.*, 1889, ii, 1182.

Takayasu, R., Über die Beziehungen zwischen anatomischen Glomerulusveränderungen und Nierenfunktion bei experimentellen Nephritiden, *Deutsch. Arch. klin. Med.*, 1907–08, xcii, 127.

Volhard, F., and Fahr, T., Die Brightsche Nierenkrankheit; Klinik. Pathologie und Atlas, Berlin, 1914, 34.

Wade, W. F., On diphtheritis, *Midland Quart. J. Med. Sc.*, 1858, ii, 169, 390; Notes upon diphtheria, *Lancet*, 1862, ii, 203.

Wagner, E., Beiträge zur Kenntniss des acuten Morbus Brightii, *Deutsch. Arch. klin. Med.*, 1879–80, xxv, 529 (see especially pp. 545–547).

Welch, W. H., and Flexner, S., The histological changes in experimental diphtheria. Preliminary communication, *Bull. Johns Hopkins Hosp.*, 1891, ii, 107.

EXPLANATION OF PLATES.

PLATE 9.

FIG. 1. Rabbit 7. Leitz obj. 6. Dosage 0.0014 cc. of toxin per kilo. Glomerulus showing marked increase of endothelial nuclei. The tubules are practically normal.

FIG. 2. Rabbit 7. Oil immersion. Swollen endothelial cells in glomerulus. The capillaries are still patent.

FIG. 3. Rabbit 14. Leitz obj. 6. Dosage 0.0024 cc. per kilo. Dilated empty glomerular capillaries.

FIG. 4. Rabbit 15. Leitz obj. 6. Dosage 0.004 cc. per kilo. Large intraglomerular hemorrhage. Erythrocytes and desquamated epithelium in capsular space, and erythrocytes in proximal convoluted tubule (to left).

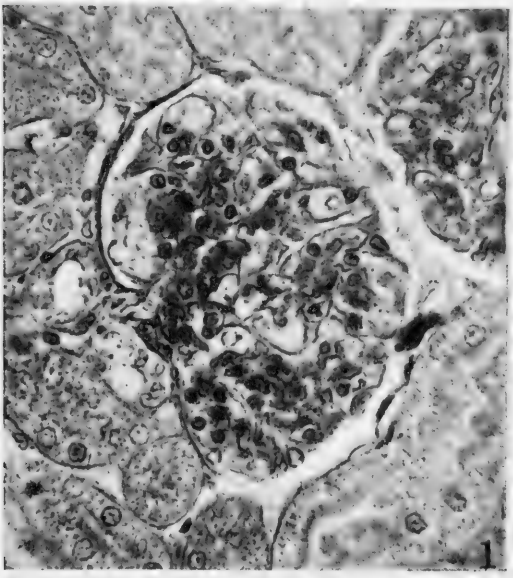
PLATE 10.

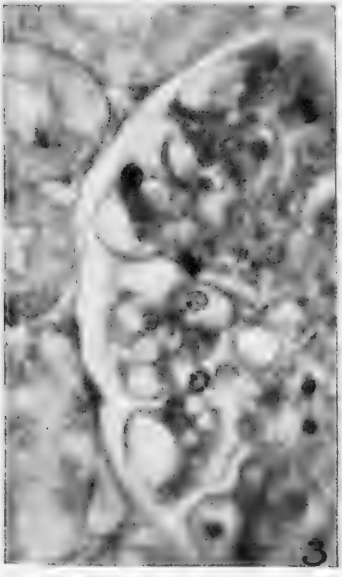
FIG. 5. Rabbit 16. Leitz obj. 6. Dosage 0.0065 cc. per kilo. Karyorrhexis and pyknosis of nuclei of tubules. The epithelium is beginning to desquamate into the lumen. Nuclei found between the tubules are also pyknotic.

FIG. 6. Rabbit 16. Leitz obj. 3. Afferent to a glomerulus containing masses of fibrin. The nuclei throughout the field are pyknotic.

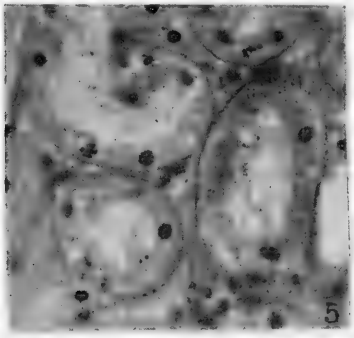
FIG. 7. Rabbit 19. Leitz obj. 6. Dosage 0.0067 cc. per kilo. Tubules showing desquamation and beginning cast formation. Several nuclei show pyknosis or fragmentation.

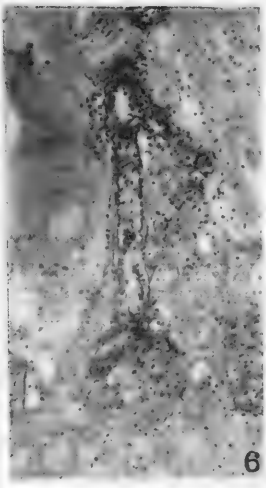
FIG. 8. Rabbit 22. Leitz obj. 6. Dosage 0.0141 cc. per kilo. The endothelium is swollen and necrotic, obliterating the capillary lumina. The nuclei are pyknotic and misshapen. The capsular space is filled with a homogeneous serous exudate. The epithelium of the capsule (parietal layer) is *in situ* and apparently little affected.

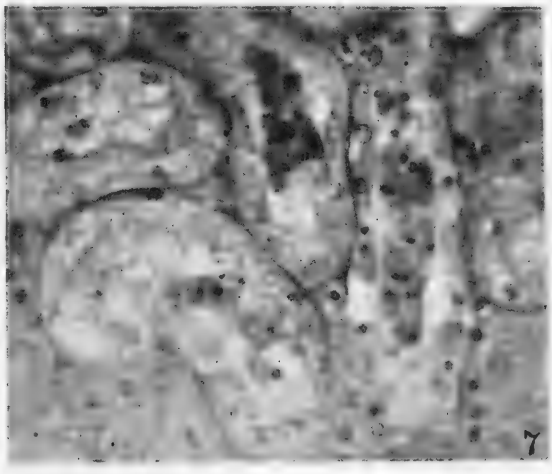


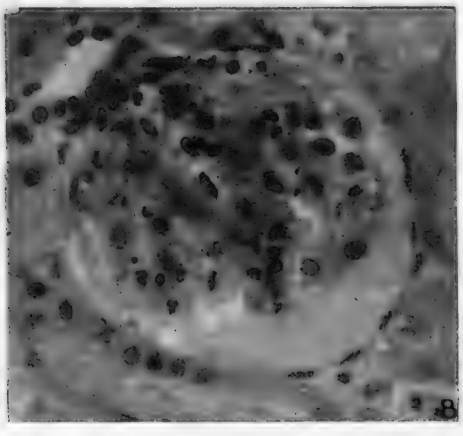


(Faber: Studies in glomerulonephritis I.)









(Faber. Studies in glomerulonephritis I.)

STUDIES IN GLOMERULONEPHRITIS.

II. A Form of Acute Glomerulonephritis Produced with Diphtheria Toxin and Bacillus coli.

By HAROLD KNIEST FABER, M.D.

(From the Laboratory of Pediatrics of Stanford University Medical School, San Francisco.)

Plates 11 and 12.

(Received for publication, March 16, 1917.)

In a previous communication it was stated that a type of glomerular injury corresponding in a general way to the intracapillary form of glomerulonephritis of Volhard and Fahr was produced by intravenous injections of diphtheria toxin and that the grade of injury was proportional to the amount of toxin introduced. Since, as a result of this knowledge, it is possible to estimate the amount of injury which can be expected to follow the injection of a given amount of toxin, it was believed that the effect of living pathogenic bacteria on a kidney already damaged to a known extent by toxin could also be correctly estimated, and that such a study might throw light on the relationship of infection to glomerulonephritis. It will be shown in a future communication that it was found impossible to produce marked glomerular lesions with bacteria alone.

EXPERIMENTAL.

In the following experiments *Bacillus coli communior* was selected because it is highly pathogenic for rabbits and belongs to that group of organisms which evince a special susceptibility to lysis. From our knowledge of this group we appear to be justified in ascribing a part, at least, of the pathogenic action to the poisonous groups split from their proteins during bacteriolysis.

TABLE I.

Series A. Injection of Diphtheria Toxin and of Colon Bacillus.

Rabbit No.	Dose of toxin / Weight	Dose per kilo.	Interval.	Dose of colon bacilli.	Died or killed.	Length of life after second injection.	Combined weight of kidneys.	Renal lesions.
		cc.	*days*	*cc.*			*gm.*	
1*	0.004 / 1300	0.0031	3	0.1	D.	36 hrs.	17.0	Many recent intraglomerular hemorrhages. Great leukocytic infiltration of tufts. Occasional masses of red blood cells in tubules. Tubular epithelium nearly normal.
2*	0.004 / 1200	0.0033	3	0.1	"	7 days.	16.4	Large rings and crescents of fibrin in capsular spaces and between loops in the tufts. Red blood cells in masses in a few capsular spaces. Moderate epithelial degeneration of tubules.
3*	0.005 / 1250	0.004	1	0.1	K.	6 "	19.0	Many intraglomerular hemorrhages and fibrin masses. Extreme capsular exudate, fibrin, albumin, and red blood cells. Hyaline and blood casts. Only slight tubular changes.
4*	0.005 / 1250	0.004	3	0.1	D.	48 hrs.	13.5	Many recent hemorrhages and numerous leukocytes in tufts. Some serous and cellular exudate in capsular spaces. Slight tubular degeneration.
5*	0.005 / 1100	0.0045	3	0.1	"	12 "	9.8	Glomeruli, swelling of endothelium, many leukocytes, especially in peripheral loops, much granular exudate, and occasional red blood cells in capsular space.

6	$\frac{0.001}{1200}$	0.0008	1	0.1	K.	4 days.	12.9	Wedge-shaped infarcts surrounded by zone of hemorrhage and leukocytic infiltration. Glomeruli, some large with empty loops; some contain intraglomerular hemorrhages Some show fibrin, desquamated epithelium, red blood cells, and white blood cells in capsular space. Many hyaline casts, some giving hemoglobin stain.
7	$\frac{0.001}{750}$	0.0013	1	0.01	D.	20 min.	7.0	No striking abnormalities.
8	$\frac{0.001}{750}$	0.0013	1	0.01	"	4 days.	12.0	Extreme reaction. Masses of fibrin or fibrin and red blood cells in the tufts and capsular space. Some fresh hemorrhages. Many hyaline casts. Many leukocytes in the tufts. Occasional serous exudate in capsular space.
9	$\frac{0.002}{1300}$	0.0015	1	0.1	"	3 "	18.9	Intraglomerular hemorrhages. Red and white blood cells and desquamated epithelium in capsular space. Many hyaline casts staining for hemoglobin, many of them containing red blood cells. Glomeruli large. Very marked spontaneous nephritis.
10	$\frac{0.003}{1400}$	0.0021	1	0.1	"	9 "	14.0	Fresh and organized hemorrhages in tufts. Fibrin, serum, red blood cells, and desquamated epithelium in capsular spaces. Half-moons. Very marked leukocytic infiltration of tufts. Frequent epithelial degeneration of tubules. Many hyaline casts.

* Older, somewhat deteriorated sample of toxin. Doses of 0.007 and 0.01 cc. per kilo failed to cause hemorrhages.
† All doses of bacilli are given in cubic centimeters of a 24 hour broth culture.

TABLE II.

Controls to Series A. Injection of Colon Bacillus.

Rabbit No.	Amount of culture injected.	Weight.	Died or killed.	Length of life after injection.	Renal lesions.
		gm.			
11	2 agar slants.	1,500	D.	1½ hrs.	No notable changes.
12	6 cc. of broth culture.	1,500	"	22 "	Considerable excess of leukocytes in tufts. Slight endothelial swelling. No exudate in capsular space.
13	4.5 cc. of broth culture.	1,400	"	36 "	An occasional erythrocyte and desquamated epithelial cell in capsular space. Slight endothelial swelling. A few hyaline casts.
14	3 cc. of broth culture.	1,250	"	5 days.	Moderate endothelial swelling with occasional empty glomerular loops. No exudate.
15	2 cc. of broth culture.	1,450	"	2 hrs.	Marked congestion of glomeruli. No other notable changes.
16	0.1 cc. of broth culture.	1,100	K.	2 days.	No notable changes.

Series A. An Injection of Diphtheria Toxin Followed by One of Bacillus coli.

An injection of diphtheria toxin was given 1 to 3 days before the bacteria. The toxin used in this and the next series of experiments was partly from the same sample used in the earlier series in which the effect of toxin alone was determined, and partly from a sample which, though originally of a strength of 175 minimum lethal doses per cc. for guinea pigs, had deteriorated so much that at the time of these experiments it had less than half the strength of the first, as shown by controls. The colon bacilli were given intravenously in doses usually of 0.1 cc. of a 24 hour broth culture. This dosage represented about one-fifth of the minimum lethal dose. The details of the experiments are given in Table I and of the controls in Table II.

The changes were different quantitatively, and also qualitatively from those due to diphtheria toxin alone. A much greater fibrin production is the most striking difference, and on close study it becomes evident that a true fibrinous exudate has occurred in the capsular space from the visceral layer of Bowman's capsule. Associated with the fibrin are seen in varying numbers red blood cells (not constant) and desquamated epithelial cells, the latter derived from the visceral layer, the parietal layer usually appearing to be intact except in occasional glomeruli, where stratification has taken place and the formation of half moons begun. Within the glomeruli, wedge-shaped and circular masses of fibrin are of frequent occurrence. In rabbits killed before these changes are fully developed, a clear, serous exudate is found in the capsular space, accompanied by varying numbers of red cells, while within the tufts globular hemorrhages are sometimes seen. Leukocytes are found, often in large numbers in the neighborhood of the fibrin masses, usually inside the tuft, but also occasionally in the capsular space. Many hyaline and occasional blood casts are present in all the kidneys.

Series B. An Injection of Vaughan's Split Protein (Poisonous Part) of the Colon Bacillus[1] *Following an Injection of Diphtheria Toxin.*

The results of this procedure are shown in Table III.

The renal changes which occurred in the rabbit (No. 20) which received the largest dose of split protein and reacted most severely will be given in detail.

Rabbit 20.—The kidneys are deep red and intensely congested throughout. The cortical surface shows a fine, red mottling and occasional pin-point hemorrhages. On the cut surface the rays are seen to be deeply congested, while the cortical surface between them is grayish and swollen. An occasional pin-point hemorrhage in the cortex is seen. The medulla is also deeply congested. With Van Gieson's stain the glomeruli are found to be very large, some of them herniating into the proximal tubule, and many of them contain globular hemorrhages. A few show large collections of red blood cells in the capsular spaces and in others

[1] I am indebted to Dr. V. C. Vaughan for a supply of split protein (poisonous part) of colon bacillus which he kindly had prepared for me.

TABLE III.

Series B. Injection of Diphtheria Toxin and of Colon Bacillus Split Protein.

Rabbit No.	Dose of toxin / Weight	Dose per kilo.	Interval.	Dose of colon bacillus split protein.	Died or killed.	Length of life after second injection.	Combined weight of kidneys.	Renal lesions.
		cc.	*days*	*gm.*			*gm.*	
17	0.002 / 1475	0.0014	2	0.07	K.	2 days.	11.0	Glomeruli congested. Occasional red blood cells in capsular space. Blood casts and hyaline casts stained with hemoglobin, mostly in ascending loop of Henle. No leukocytic reaction.
18	0.002 / 1200	0.0017	2	0.07	"	2 "	12.1	Occasional red blood cells in capsular space and tubules. No hemorrhages or exudate. Spontaneous nephritis.
19	0.002 / 1360	0.0015	2	0.08	"	2 "	13.0	A few glomeruli show a hemoglobin-tinted clear exudate in capsular space extending down into tubules. Occasional red blood cells in capsular space. A few small hemorrhages in the tufts. Desquamated epithelium in capsular space.
20	0.002 / 1425	0.0014	2	0.1	"	2 "	15.0	Marked glomerular changes. Abundant exudate. (See protocol.)
21	0.002 / 1375	0.0015	2	0.15	D.	15 min.	11.7	No notable changes.
					Controls.*			
	Weight. *gm.*							
22	1,230			0.1	K.	17 hrs.		No notable changes in either kidney.
23	1,230			0.1	"	17 "		" " " " " "
24	1,400			0.1	"	18 "		" " " " " "
25	1,500			0.1	"	18 "		" " " " " "
26	1,480			0.1	"	19 "		" " " " " "

* All injections in the controls were into the right renal artery.

merely isolated erythrocytes. In the capsular space of a few glomeruli there is a homogeneous, transparent material which takes the characteristic stain for hemoglobin. This material is more abundant in the proximal tubules and is also found in various other parts of the uriniferous tubules, as hyaline, yellow-staining casts. In several glomeruli there is marked epithelial desquamation. There is no increase of leukocytes either in the tufts or in the capsular space. The epithelium of the tubules, especially of the proximal tubules, is beginning to desquamate and the nuclei in many places stain faintly. There is a slight increase of the cells of the glomerular endothelium. Similar changes, though less intense, were present also in the kidneys of Rabbits 17 and 19, which received smaller doses of split protein. It may be pointed out that they did not occur in Rabbit 21, which died 15 minutes after the inoculation.

A few experiments which gave negative or inconclusive results may be briefly mentioned.

Rabbit 27.—Weight 1,450 gm. Received 0.001 gm. of uranium acetate intravenously. 6 days later received 0.5 cc. of a 24 hour broth culture of *B. coli communior*, and died in about 12 hours. The kidneys showed an extreme serofibrinous exudate in the capsular spaces of a majority of the glomeruli, and also many large hemorrhages within the tufts, besides the usual tubular degeneration of uranium nephritis.

Four other rabbits similarly treated failed to show these glomerular changes.

Rabbits 28 and 29, weighing respectively 1,600 and 1,500 gm., received 0.001 gm. of uranium acetate intravenously and after an interval of 4 days received 0.5 cc. of a 24 hour broth culture of *Streptococcus viridans*. No glomerular lesions were found.

DISCUSSION.

Compared with the renal lesions following diphtheria toxin alone, those following successive inoculations of diphtheria toxin and colon bacilli show marked differences. The latter are characterized by a striking tendency to exudation, in the earlier stages, of a clear serous fluid containing small amounts of fibrin and varying amounts, often large, sometimes small, of red blood cells in the capsular space, hemorrhages into the tufts after doses of toxin which alone would not cause hemorrhage, and a fairly constant leukocytic infiltration of the glomeruli. Hyaline casts sometimes containing red blood

cells and occasionally taking the yellow stain of hemoglobin are of frequent occurrence. At a later stage large masses of fibrin in crescents and rings are found on the visceral layer of Bowman's capsule (Figs. 1, 4, 5, and 6), associated with desquamated epithelial cells, occasional erythrocytes and leukocytes, the last named being, however, infrequent in this location. The peripheral layer of the capsule shows in places beginning stratification forming incipient half moons (Figs. 2 and 3). Within the tufts, wedge-shaped or round masses of fibrin are seen which are commonly surrounded by a zone of leukocytic infiltration (Fig. 2).

The changes found after successive injections of diphtheria toxin and Vaughan's split protein of the colon bacillus (poisonous part) also show a marked tendency to exudation (Fig. 7). The capsular spaces of a majority of the glomeruli, when a sufficient amount of split protein has been given, contain an abundant serous fluid stained with hemoglobin which can be traced down the tubules as casts. Red blood cells are sometimes found in the capsular spaces; in the tubules they are frequently embodied in the casts. Intraglomerular hemorrhages occur, though not so commonly as after injections of colon bacilli. A leukocytic reaction was not observed. The later stages of reaction to this type of injury were not studied.

On the whole, the correspondence between the early renal lesions of the two series is close. The main point of dissimilarity, the leukocytic reaction occurring in the first series and absent in the second, admits of explanation as phagocytosis provoked by solid bacterial particles but not by bacterial substance in solution. The appearance of laked hemoglobin in the exudate was more marked in the second series, but was observed occasionally also in Rabbit 3 of the first series.

It seems likely from a study of the two sets of experiments that the lesions in both were essentially of the same nature and it is believed that the mechanism in both cases was the same. The effect of the colon bacillus, in other words, was due to the poisonous molecular groups liberated from its protein by the lytic activities of the body. One must, however, be cautious in making a wide application of this conclusion since in control animals it was not found possible to produce marked renal lesions with repeated injections of colon bacilli

alone or with large amounts of split protein injected directly into the renal artery. Preliminary injury seems to be requisite.

It is interesting to find that the renal lesions of the present experiments correspond closely with those described in man by Volhard and Fahr as the "extracapillary form of glomerulonephritis." The same exudate of fibrin and cells in the capsular space, the same masses of fibrin within the tuft, and the same stratification of the capsular epithelium occur in both.

Without fully agreeing with Ophüls that a distinction between these two forms of glomerulonephritis is "an unnecessary refinement" it seems probable that the extracapillary form reflects a later and severer grade of renal irritation with, perhaps, the addition of a specific reaction of the epithelial elements of the tuft, and from the present experiments it would appear that infection, acting through the poisonous groups split from the protein molecules of bacteria by some lytic reaction in the hosts, is capable of intensifying what was originally a minor grade of injury and thus of producing the severer lesions characteristic of the extraglomerular type.

SUMMARY.

1. Rabbits in which a mild grade of glomerular damage has been produced by small doses of diphtheria toxin develop a severe glomerulonephritis corresponding to the extracapillary form described by Volhard and Fahr after inoculations with sublethal quantities of *Bacillus coli*.

2. Similar, though not completely identical lesions are produced when diphtheria toxin is followed by intravenous injections of Vaughan's split protein (poisonous part) of *Bacillus coli*.

3. The inference is drawn that under the experimental conditions described *Bacillus coli* undergoes bacteriolysis and produces glomerular lesions through the toxic groups contained within its protein molecule.

BIBLIOGRAPHY.

Faber, H. K., Studies in glomerulonephritis. I. A quantitative study of the reaction of the kidney to diphtheria toxin, *J. Exp. Med.*, 1917, xxvi, 139.

Ophüls, W., Nephritis. A new series of cases with a review of recent literature, *J. Am. Med. Assn.*, 1915, lxv, 1719.

Volhard, F., and Fahr, T., Die Brightsche Nierenkrankheit, Berlin, 1914. See pp. 33–35.

EXPLANATION OF PLATES.

PLATE 11.

FIG. 1. Rabbit 8. Leitz obj. 3. Dosage 0.0013 cc. of toxin per kilo followed by 0.01 cc. of colon bacillus broth. Acute glomerulonephritis. The capsular spaces are filled with fibrin and the tufts are compressed.

FIG. 2. Rabbit 8. Leitz obj. 6. A wedge-shaped fibrin mass, *a*, in the upper glomerulus is surrounded by infiltrated leukocytes, *b*. In the lower glomerulus the capsular epithelium is stratified forming a half moon, *c*.

FIG. 3. Rabbit 8. Leitz obj. 6. Leukocytic infiltration of the glomerulus, *a*, and stratification of the parietal epithelium of the capsule, *b*, are shown.

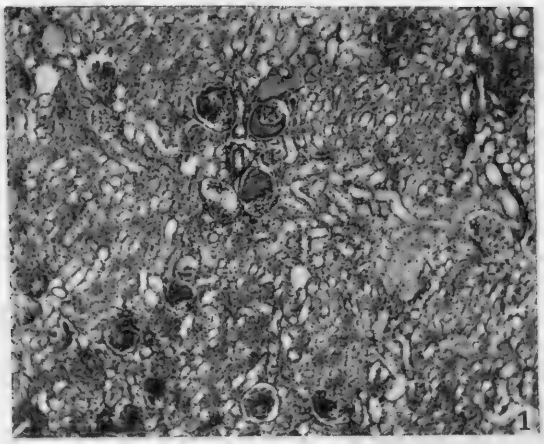
PLATE 12.

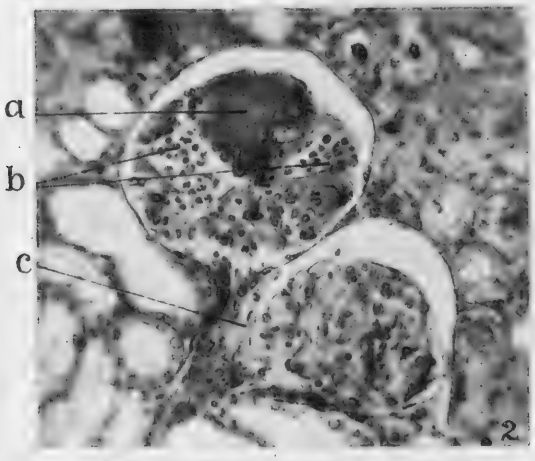
FIG. 4. Rabbit 10. Leitz obj. 6. Dosage 0.0021 cc. of toxin per kilo followed by 0.1 cc. of colon bacillus broth. Enormous dilation of the capsular space, which contains an albuminous exudate, a crescent of fibrin, some desquamated epithelium, and the remnants of the glomerulus, is shown.

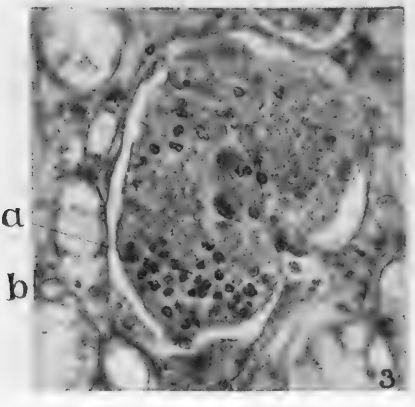
FIG. 5. Rabbit 2. Leitz obj. 6. Dosage 0.0033 cc. of toxin (old) followed by 0.1 cc. of colon bacillus broth. The glomerulus is entirely surrounded by a ring of fibrin. The capsular space is greatly dilated but empty. The capsular epithelium is peeling.

FIG. 6. Rabbit 2. Leitz obj. 6. The glomerulus has a thick cap of fibrin outside of which in the dilated capsular space are masses of red blood cells. The parietal layer of the capsule is beginning to separate.

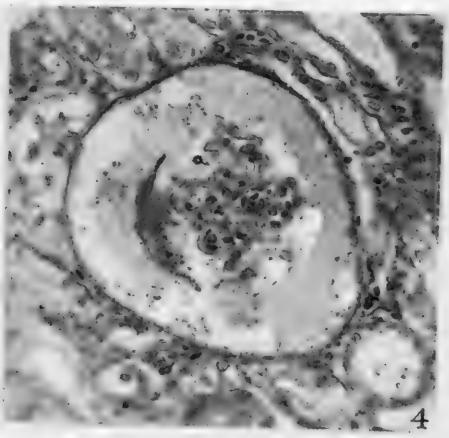
FIG. 7. Rabbit 20. Diphtheria toxin followed by 0.1 gm. of split protein of the colon bacillus. The glomerulus contains a fresh hemorrhage and the capsular space is filled with a transparent serous exudate which, mixed with granular debris, extends into the neck.



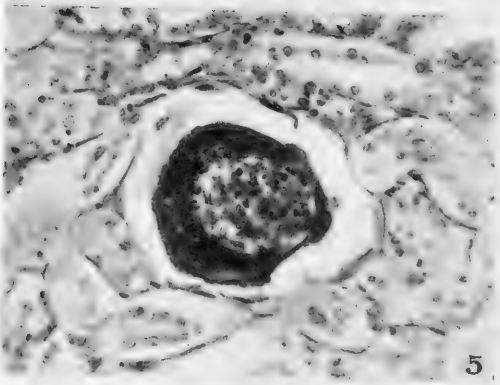




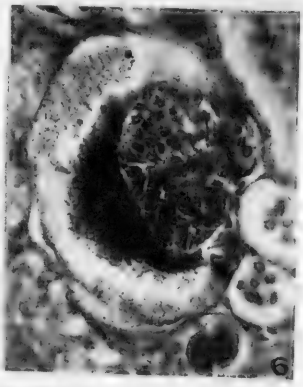
(Faber: Studies in glomerulonephritis. II.)



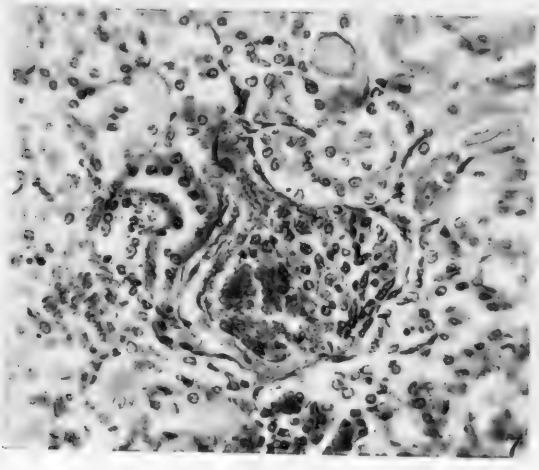
4



5



6



7

(Faber: Studies in glomerulonephritis. II.)

EXPERIMENTAL STUDIES UPON LYMPHOCYTES.

II. The Action of Immune Sera upon Lymphocytes and Small Thymus Cells.

By ALWIN M. PAPPENHEIMER, M.D.

(*From the Department of Pathology of the College of Physicians and Surgeons, Columbia University, New York.*)

Plate 13.

(Received for publication, February 15, 1917.)

In a recent paper[1] brief reference was made to the production of cytotoxic sera for lymphocytes derived from human tonsils and from the rat thymus. It is desired to report here upon further experiments which have been carried out with these sera, and which appear to bear directly upon the general problem of the specificity of cytotoxins, and upon the important question of the biological identity of the small thymus cells with the lymphocytes found in the lymphoid tissues and in the circulating blood.

The method used in these studies has been described in detail in a previous paper. Briefly stated, it consists in subjecting suspensions of thymus or tonsil cells in salt solution or Locke's fluid for a given period, to the action of whatever toxic agent is chosen, and then adding trypan blue in appropriate dilution. The percentage of diffusely stained cells affords a quantitative measure of the injury produced, when compared with a control suspension maintained under similar conditions. The factors of error, and the precautions necessary in order to make the determinations of value are related in the previous paper.

Brief reference should be made to the more important studies of previous workers, using other methods.

Metchnikoff[2] in 1899 induced leukotoxic sera by injecting spleen and lymph node emulsions of rat and guinea pig into rabbits. He observed the agglutinative

[1] Pappenheimer, A. M., The reactions of lymphocytes under various experimental conditions, *J. Exp. Med.*, 1917, xxv, 635.

[2] Metchnikoff, E., Études sur la résorption des cellules, *Ann. Inst. Pasteur*, 1899, xiii, 737.

and lytic changes which followed when suspensions were exposed to the action of the immune serum, and described the hydropic swelling of the mononuclears "by which they become transformed into transparent vesicles, the nucleus thereby being rendered very visible." He found that his sera were toxic only for the cells of the species used as antigen, but that there was no evidence of specificity for any particular type of leukocyte, polymorphonuclears and mononuclears being equally affected by anti-lymph-gland serum.

Besredka[3] continuing these studies found that the toxic properties of the leukotoxic sera were destroyed by heating to 55°C. for 30 minutes, and that emulsions of cells heated to 60°C. lost their antigenic properties. He also observed that his sera were mildly hemolytic, although the injected suspensions were macroscopically blood-free. Injections of leukotoxic sera into normal animals produced toxic, or, in large doses, lethal effects. The blood showed an initial hypoleukocytosis followed by hyperleukocytosis.

Flexner[4] has contributed to the subject a detailed study of the lesions produced in the hematopoietic tissues by myelotoxic and lymphotoxic sera. In the lymph glands the principal changes produced were hyperplasia of the follicles, swelling of the germinal centers, and degeneration of the large cells in the center of the follicles. The degenerative changes in general, however, are described as "minimal and trifling."

Bunting,[5] continuing the work of Flexner, immunized geese with lymph glands and bone marrow of the rabbit, and obtained sera which were "to a large extent specific both in their action on the tonsils and on the circulating blood." One of the sera of the two geese used as controls, however, showed well marked hemolytic powers, and both were agglutinative and lytic for suspensions of lymph gland cells *in vitro*. This action was, however, less intense than that of the serum from a goose immunized against lymph glands.

Christian and Leen[6] have used the cessation of motion of leukocytes observed in a warm chamber as an index of toxicity. They found that sera having both hemolytic and leukotoxic properties could be produced by immunizing with a variety of somatic cells, as those of the liver, spleen, kidney, and cardiac muscle.

The production of thymotoxic sera has been attempted by Moorhead[7] and by

---

[3] Besredka, La leucotoxine et son action sur le système leucocytaire, *Ann. Inst. Pasteur*, 1900, xiv, 390.

[4] Flexner, S., The pathology of lymphotoxic and myelotoxic intoxication, *Univ. Penn. Med. Bull.*, 1902, xv, 287.

[5] Bunting, C. H., The effects of lymphotoxins and myelotoxins on the leucocytes of the blood and on the blood-forming organs, *Univ. Penn. Med. Bull.*, 1903–04, xvi, 200.

[6] Christian, H. A., and Leen, J. F., Some further observations on leucocytotoxins, *Boston Med. and Surg. J.*, 1905, clii, 397.

[7] Moorhead, T. G., The thymus gland, *Practitioner*, 1905, lxxv, 733.

Ritchie.[8] Moorhead states briefly that sera from rabbits immunized with guinea pig thymus did not agglutinate thymus cells *in vitro*, and had no leukolytic action. Ritchie, however, using complement fixation methods found that the sera of ducks immunized with suspensions of guinea pig thymus contained an immune body having an affinity for the receptors of the guinea pig thymus and lymph glands, spleen, and bone marrow. In the presence of this immune body, guinea pig complement became fixed, so that there was no hemolysis in the hemolytic system used as a test. The sera showed no affinity for the receptors of the liver, adrenal, thyroid, lung, etc. The serum was not hemolytic, and its binding powers were not affected by heating.

The cytotoxic sera were prepared by injecting rabbits intravenously with washed suspensions of rat thymus cells or of tonsil lymphocytes. The former could be obtained almost blood-free by exsanguinating the rat, carefully dissecting off the superficial blood vessels from the gland, and washing the suspended cells in one or more changes of salt solution. The tonsil suspensions were usually more or less admixed with red blood cells.

EXPERIMENTAL.

The following experiments illustrate the cytotoxic and cytoagglutinative properties of these sera, when tested against the appropriate antigen.

Experiment 1.—Dec. 5, 1916. Serum C (normal control), Serum H (immunized against human tonsil lymphocytes), and Serum R (from rabbit immunized against rat thymus cells) were added in the proportion of 1 : 25 to a suspension of rat thymus cells. The tubes were then placed in the incubator for 35 minutes, trypan blue was added in 1 : 5,000 dilution, and the percentage of stained cells determined. Duplicate counts were made.

Mixture.	Stained cells.	Unstained cells.	Percentage of stained cells.	Agglutination.
Serum C, 0.02 cc.	195	417	31.8	0
Thymus cells, 0.5 "	212	469	31.1	
Serum H, 0.02 "	228	208	52.3	0
Thymus cells, 0.5 "	322	329	49.5	
Serum R, 0.02 "	309	243	55.9	++
Thymus cells, 0.5 "	284	194	59.4	++

[8] Ritchie, W. T., The specificity and potency of adrenolytic and thymolytic sera, *J. Path. and Bacteriol.*, 1908, xii, 140.

Although the highest count is obtained with Immune Serum R all the tubes show a high proportion of stained cells. Agglutination occurred only in Serum R.

The sera were then diluted 1:10 and inactivated for 30 minutes at 58°C. A fresh suspension of thymus cells from a healthy young rat was prepared and the following tubes were set up and incubated for 15 minutes at 37°C.

Mixture.	Stained cells.	Unstained cells.	Percentage of stained cells.	Agglutination.
Serum C, 0.5 cc. Thymus cells, 0.2 "	17	473	3.4	0
Serum H, 0.5 " Thymus cells, 0.2 "	38	433	8.0	0
Serum R, 0.5 " Thymus cells, 0.2 "	27	438	5.8	++

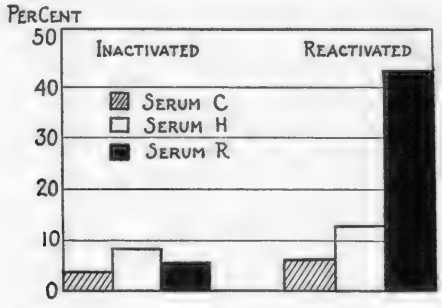
The toxicity of the serum is thus destroyed by heating. Agglutination by Serum R is still marked.

To each tube was added 0.5 cc. of fresh 1:10 guinea pig complement and the tubes were replaced in the thermostat. Counts were made after 1 hour and 15 minutes.

Mixture.	Stained cells.	Unstained cells.	Percentage of stained cells.	Agglutination.
Serum C, 0.5 cc. Thymus cells, 0.2 " Complement, 0.5 "	30	515	5.5	0
Serum H, 0.5 " Thymus cells, 0.2 " Complement, 0.5 "	60	427	12.3	0
Serum R, 0.5 " Thymus cells, 0.2 " Complement, 0.5 "	113	150	42.9	+++

Under the influence of the immune serum the thymus cells are markedly agglutinated, so that there are comparatively few free cells. They are highly irregular in shape, and a great proportion of them are diffusely stained with trypan. Some of the cells show a hydropic swelling of the cytoplasm, the stained nucleus appearing suspended in a clear vesicle. The control sera produced no such effect. The results are shown graphically in Text-fig. 1.

Numerous experiments have given uniform results. Serum R was last tested on January 30, 1917, 26 days after the rabbit had received an injection of thymus cells. The toxicity and agglutinative power were even more marked than in the experiment cited.



TEXT-FIG. 1. Experiment 1. The effect of Thymotoxic Serum R upon the stainability of rat thymus cells.

The following protocol illustrates a similar action of the serum from Rabbit H, immunized with a suspension of human tonsil lymphocytes, when tested against the appropriate antigen.

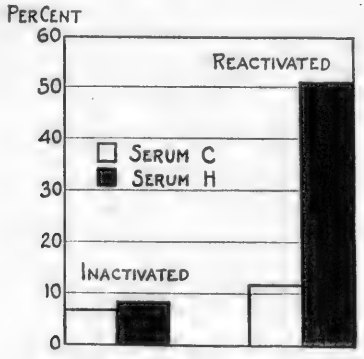
Experiment 2.—Dec. 12, 1916. Serum from Rabbit H, which had received four intraperitoneal and two intravenous injections of human tonsil lymphocytes, and Serum C from a control normal rabbit were tested against suspensions of human lymphocytes. The suspension, twice washed and centrifuged, had stood over night in the ice box. Sera were inactivated for 30 minutes at 58°C. and diluted to 1:10.

Mixture.	Stained cells.	Unstained cells.	Percentage of stained cells.	Agglutination.
Serum H (1:10), 1.0 cc. Tonsil lymphocytes, 0.2 "	26	283	8.4	+
Serum C (1:10), 1.0 " Tonsil lymphocytes, 0.2 "	27	363	6.9	0

0.5 cc. of a 1:10 dilution of fresh guinea pig complement was added and the tubes were replaced in the thermostat. Counts made after 45 minutes showed the following:

Mixture.	Stained cells.	Unstained cells.	Percentage of stained cells.	Agglutination.
Serum H (1:10), 1.0 cc.	163	156	51.0	++
Tonsil lymphocytes, 0.2 "				
Serum C (1:10), 1.0 "	40	273	12.7	0
Tonsil lymphocytes, 0.2 "				

There is thus a striking increase in the proportion of stained cells under the influence of the immune serum, accompanied by macroscopic agglutination, and the morphological changes described above. The results are shown in Text-fig. 2.



TEXT-FIG. 2. Experiment 2. The effect of lymphocytoxic serum upon the stainability of human tonsil lymphocytes.

Repeated observations having assured us of the constancy of this cytotoxic action of Anti-thymus Serum R, the effects produced *in vitro* were compared with those which might be caused by injection into the living animal.

Experiment 3.—Dec. 14, 1916. Two young rats were etherized and a fragment of thymus was removed from each for histological control. Sections showed a normal structure.

Dec. 19. Rat A was injected intraperitoneally with 1 cc. of native Anti-thymus Serum R. The control, Rat B, received normal rat serum. After 24 hours the rats were killed and suspensions in Locke's fluid were made from a portion of each thymus gland, the remainder being fixed and sectioned. Counts of the suspensions, with and without the addition of rat serum, showed no significant

differences which would indicate an increased fragility on the part of the cells of the rat which had received the cytotoxic serum. Correspondingly, no histological changes were found in sections of the tissue, as compared with the control. The experiment, therefore, was negative.

Experiment 4, however, in which the sera were first inactivated and complement was injected simultaneously gave definite indication of cytotoxic action *in vivo*, and also confirms the validity of the method used in determining cell injury.

Experiment 4.—Dec. 21, 1916. Rats A and B, well nourished young animals of approximately the same age and weight, were etherized and fragments of thymus excised from each. Portions of the fragments were fixed in Zenker's fluid, sectioned, and found to show a normal structure. The remainder was teased in Locke's solution and counts were made in the usual manner.

Mixture.	Stained cells.	Unstained cells.	Percentage of stained cells.
Rat A thymus cells in Locke's fluid.....	30	551	5.1
" B " " " " "	44	442	9.0

Dec. 26. Wounds healing and uninfected; rats in good condition. Rat A received 1.5 cc. of Anti-thymus Serum R (inactivated) and 1 cc. of fresh guinea pig complement (1:10) intraperitoneally. Rat B received 1.5 cc. of Serum C (normal rabbit inactivated) and 1 cc. of fresh guinea pig complement (1:10) intraperitoneally.

Rats killed after 24 hours. The thymus of each was divided into two parts; one was used for determining the stainability of cells, the other fixed and sectioned. The count shows the following, after 1 hour at 37°C.

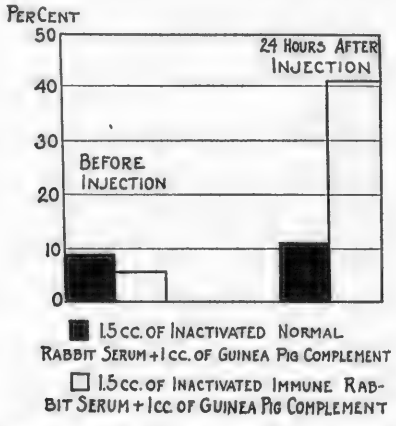
Mixture.	Stained cells.	Unstained cells.	Percentage of stained cells.
Rat A thymus cells in 0.85 per cent salt solution.....	112	156	41.7
Rat B thymus cells in 0.85 per cent salt solution.....	58	424	12.0

The cells of Rat A, which before the injection of cytotoxic serum were somewhat more resistant than those of Rat B, have now been rendered much more fragile, as shown by the greatly increased number of stained cells (Text-fig. 3).

The study of the histological preparations indicates that the thymus of Rat A has been severely damaged by the cytotoxic serum

(Fig. 1). The gland which macroscopically was smaller and firmer than the control shows a great rarefaction of the cortex, fragmentation of the small thymus cells, and clumps of bluish staining material (remains of altered chromatin) in and amongst the conspicuous reticular cells. The interlobular septa are edematous and contain wandering cells of different types. There are no hemorrhages.

There is thus a convincing correspondence between the lesions produced and the increased fragility of the cells under the influence of the immune serum *in vitro*. The negative results of the previous experiments, in which the native serum was injected, are not ex-



TEXT-FIG. 3. Experiment 4. The stainability of rat thymus cells before and after the injection of immune serum.

plained. Later repetitions of this experiment have not been uniformly successful. In some instances we have observed a pronounced destruction of small thymus cells with phagocytosis of the reticular epithelium; in other cases no effects were produced. It is evident that there are variable factors which have not yet been determined. It is perhaps in accord with the well known phenomenon that, as Zinsser[9] states, "the alexin of an animal is entirely impotent or but weakly capable of producing hemolysis of the sensitized cells of its own species."

[9] Zinsser, H., Infection and resistance, New York, 1914, 154.

This possible explanation, however, was tested by comparing the complementary action of guinea pig serum and rat serum, in the presence of anti-thymus cell amboceptor and washed thymus cells. It was found that whereas 0.2 cc. of guinea pig serum was sufficient to cause a maximal cytotoxic effect in the presence of immune serum (0.5 cc.) and thymus cell suspension 0.2 cc., 0.4 cc. or double the amount of rat serum was required. Whether this slight difference in the complementary action, which was determined in only a single experiment, is a constant one, cannot be stated. It hardly affords a sufficient explanation for the negative experiment, and further studies are necessary to clear up this point.

Previous workers with leukotoxic and lymphotoxic sera have invariably found a moderate hemolytic activity upon the red cells of the species furnishing the antigen. The sera studied by us have also been moderately hemolytic, as shown by the following protocol.

Experiment 5.—Serum H from a rabbit which had been injected with human tonsil lymphocytes was tested against washed human erythrocytes.

Serum H (1 : 10) inactivated.	5 per cent red blood cells.	Salt solution.	10 per cent guinea pig complement.	Hemolysis.	Agglutination.
cc.	*cc.*				
1.0	0.5	0.5	0.5	++	+
0.5	0.5	1.0	0.5	+	0
0.1	0.5	1.4	0.5	0	0
0.05	0.5	1.45	0.5	0	0
0.01	0.5	1.49	0.5	0	0
0.00	0.5	2.00	0.5	0	0

Readings after 1 hour at 37°C. and 18 hours in the ice box showed that the serum caused slight hemolysis in a dilution of 1:50. The thymotoxic serum (R) was also feebly hemolytic for rat corpuscles.

Jan. 29, 1917. *Experiment 6.*

Serum R (1 : 10) inactivated.	5 per cent red blood cells.	Salt solution.	10 per cent guinea pig complement.	Hemolysis.	Agglutination.
cc.	*cc.*				
1.0	0.25	0.75	0.5	+	0
0.5	0.25	1.25	0.5	+	0
0.1	0.25	1.65	0.5	0	0
0.0	0.25	1.75	0.5	0	0

This brings up at once the question as to whether the thymotoxic and thymoagglutinative factors are distinct from the hemolytic ones. That the sera should be mildly hemolytic was to be expected not only from the concurrent experience of previous workers, but from the fact that the cell suspensions could not be rendered wholly blood-free even by repeated washing.

We have attacked the problem in two ways: first, by determining whether, after complete absorption of the hemolysin, the serum still retained to a degree its thymotoxic and thymoagglutinative properties; and secondly, by studying the effects upon the thymus cells of a serum prepared by immunizing a rabbit against rat erythrocytes.

Experiment 7 illustrates the fact that both thymotoxic and thymoagglutinative factors persist after complete absorption of the hemolysin and hemagglutinin.

Experiment 7.—Jan. 29, 1917. Anti-thymus Serum R from the rabbit whose last injection with thymus cells had been on Jan. 5 was inactivated. The hemolytic activity of the serum was first tested, and it was found that 0.1 cc. of serum in the presence of 0.5 cc. of 10 per cent complement completely hemolyzed 0.25 cc. of washed rat erythrocytes.

A preliminary test was also made of its cytotoxic property for thymus cells. The percentage of stained cells rose after reactivation of the serum from 6.2 to 100 per cent and agglutination was marked.

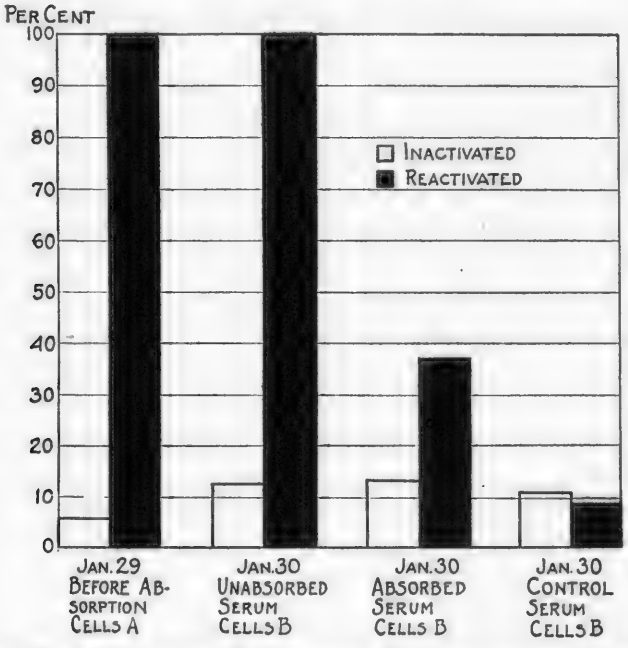
To absorb out the hemolytic factor 2 cc. of 5 per cent red cells were added to 2 cc. of inactivated serum. After 1 hour at 37°C. the red cells were removed by centrifugation, and 2 cc. of red blood cells were again added. The erythrocytes were then allowed to remain in contact with the serum in the ice box for 18 hours. On the following day the serum was pipetted off and tested both for its hemolytic activity and for its thymotoxic action.

0.1 cc. of the absorbed serum now completely failed to hemolyze 0.25 cc. of washed erythrocytes in the presence of 0.5 cc. of complement. A definite though diminished toxicity for the thymus cells could still be demonstrated, the percentage of stained cells after reactivation rising from 12.8 to 37.0 per cent. A control normal serum, tested against the same suspension, showed no increase in the percentage of stained cells, after addition of complement. These relations are shown graphically in Text-fig. 4.

Several other experiments conducted in the same manner have given comparable results. One may conclude from them that either (*a*) there is a toxic factor distinct from the hemolytic one or (*b*) the hemolytic factor persists in the absorbed serum in minimal

amount, which is impotent to cause laking of the erythrocytes, but is still sufficient so to alter the permeability of the lymphocytes that the percentage of stained cells is increased. There seems at present no way of deciding which of these two suppositions is the correct one.

In the hope of obtaining further information upon the question of the identity of hemolytic and thymotoxic substances, a hemolytic serum was prepared by injecting a rabbit with washed rat erythro-



TEXT-FIG. 4. Experiment 7. Persistence of thymotoxins and thymoagglutinins after the absorption of hemolysin from thymotoxic serum.

cytes. The toxicity of this serum for thymus cells was then tested, and the separation of the two factors by absorption with red cells and thymus cells, respectively, attempted.

Experiment 8.—Jan. 23, 1917. Serum from Rabbit H which had received four spaced injections of washed rat corpuscles intravenously was obtained 12 days after the last injection.

A. Preliminary Hemolytic Titration.

Serum (1 : 10) inactivated.	Red blood cells.	Complement.	Hemolysis.	Agglutination.
cc.	*cc.*			
1.0	0.25	0.5	+	+++
0.5	0.25	0.5	+	+++
0.25	0.25	0.5	+	++
0.12	0.25	0.5	+	+
0.0	0.25	0.5	0	0
1.0	0.25		0	0

The serum is moderately hemolytic in dilution up to 1:400 although the hemolysis is masked somewhat by the strong agglutination.

B. Preliminary Test for Thymotoxic and Thymoagglutinating Properties.

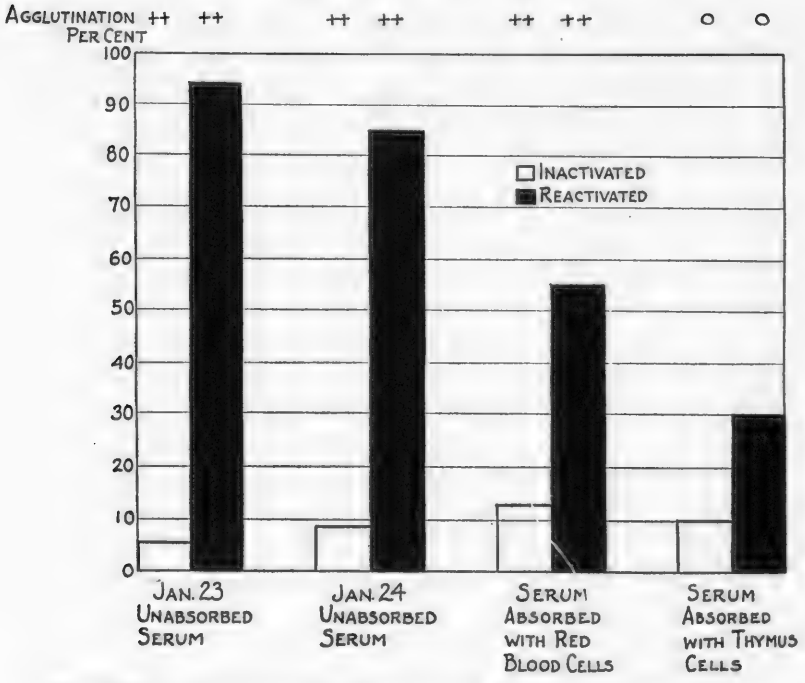
Mixture.	20 min. in incubator.			30 min. after the addition of 0.5 cc. of 10 per cent guinea pig complement.
	Stained cells.	Unstained cells.	Percentage of stained cells.	Percentage of stained cells.
Serum (1: 10) inactivated, 0.05 cc. Thymus cells, 0.1 "	27	458	5.5	94.5

C. Absorption of Hemolysin and Hemagglutinin.

Inactivated serum (1: 10).....3 cc.
5 per cent rat erythrocytes .....3 "

In the incubator 1 hour at 37°C. Centrifuged serum was pipetted off and 1 cc. of fresh suspension added. Placed in the ice box over night. The following morning the completeness of the absorption was tested. It was found that 0.1 cc. produced neither hemolysis nor agglutination of 0.25 cc. of red corpuscles in the presence of 0.5 cc. of 10 per cent complement. The absorption of the hemolysis was, therefore, apparently complete.

The toxicity of the absorbed and unabsorbed sera for rat thymus cells was then compared, a fresh suspension from a healthy young animal being taken (Text-fig. 5).



TEXT-FIG. 5. Experiment 8. Persistence of the thymotoxic factor in hemolytic serum after absorption.

Mixture.	Stained cells.	Unstained cells.	Percentage of stained cells.	Agglutination.
Absorbed serum (1:10) inactivated, 1.0 cc. Thymus cells, 0.1 cc.	40 min. in incubator.			
	73	456	13.8	−−
	45 min. after the addition of 0.5 cc. of 10 per cent guinea pig complement.			
	315	251	55.7	
Unabsorbed serum (1:10) inactivated, 1.0 cc. Thymus cells, 0.1 cc.	40 min. in incubator.			
	50	457	9.8	++
	0.5 cc. of 10 per cent guinea pig complement added. 45 min. in incubator.			
	388	67	85.3	

The serum, after apparently complete absorption of hemolysin and hemagglutinin, is still strongly agglutinative and toxic for thymus cells.

To determine whether any of the agglutinin for thymus cells had been removed by exposure of the serum to red blood corpuscles a rough quantitative comparison of the agglutinin present in the absorbed and unabsorbed serum was made.

Unabsorbed serum (1:10).			Absorbed serum (1:10).		
Serum.	Salt solution.	Agglutination.	Serum.	Salt solution.	Agglutination.
gtt.	*gtt.*		*gtt.*	*gtt.*	
10	0	+++	10	0	+++
8	2	++	8	2	+++
6	4	++	6	4	++
3	7	±	3	7	++
1	9	0	1	9	0
0	10	0			

1 drop of a thick suspension of thymus cells to each tube was used. Macroscopic readings were made after 1 hour at 37°C. There appears to be no quantitative diminution in the amount of agglutinin for thymus cells.

Experiments were undertaken to absorb out the hemagglutinative and hemolytic factors by exposing the serum to suspensions of washed thymus cells. This was done, an equal volume of a thick suspension being added, and after 1 hour in the incubator, the serum was centrifuged and pipetted off and fresh cells were allowed to stand in contact with the serum for 18 hours in the ice box.

The serum was then tested for its hemolytic and hemagglutinative powers. These were found to have been practically unaffected, 0.1 cc. upon the addition of complement still causing complete hemolysis and strong agglutination of 0.25 cc. of rat corpuscles. The thymoagglutinative and thymotoxic factors, though much reduced were still present.

To sum up this experiment, which has been repeated with similar results, one finds that (1) hemolytic serum obtained by immunizing rabbits with washed rat erythrocytes is both toxic and agglutinative for small thymus cells; (2) the thymotoxic and thymoagglutinative factors, however, cannot be completely removed by absorption of the hemolysins and hemagglutinins; (3) the hemolysin and hemagglutinin cannot be absorbed by exposure of the serum to suspensions of thymus cells.

The evidence seems to favor the view that we are dealing with two distinct factors. If we assume that the thymus cells are identical with the lymphocytes of the circulating blood, it seems probable

that specific immune bodies have been produced in response to the lymphocytes injected with the washed erythrocytes. The alternative view is the one already suggested; namely, that minute amounts of hemolysin, insufficient to cause laking of the red cells, are still able to produce the striking cytotoxic effects upon the thymus cells recognizable by the trypan blue method.

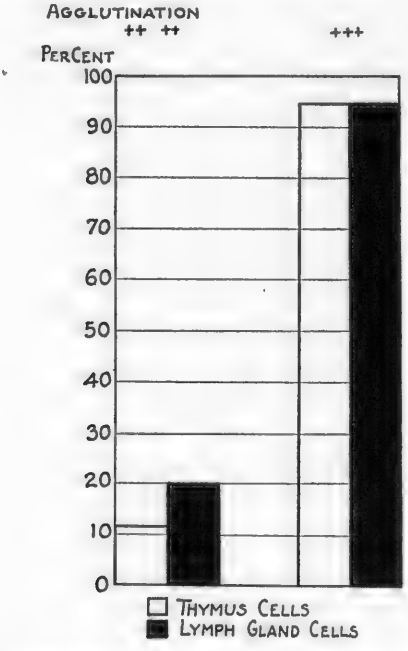
Although we have not been able to work with lymphocytes from the circulating blood, we have studied the action of thymotoxic serum upon suspensions of lymphocytes derived from lymph glands. The following illustrative protocol shows that, as far as their reaction to the immune serum is concerned, thymus cells and lymph gland lymphocytes are identical.

Experiment 9.

Jan. 30, 1917.

Mixture.	Stained cells.	Unstained cells.	Percentage of stained cells.	Agglutination.
Anti-thymus serum (1:10), 1.0 cc. Lymph gland lymphocytes, 0.2 "	55 min. in incubator.			
	112	431	20.6	— —
	45 min. after the addition of 0.5 cc. of complement.			
	493	27	94.8	
Anti-thymus serum (1:10), 1.0 cc. Thymus cells, 0.2 "	55 min. in incubator.			
	62	477	11.5	— —
	1 hr. after the addition of 0.5 cc. of complement.			
	487	25	95.1	

Both lymph gland lymphocytes and small thymus cells are severely injured after exposure to the activated anti-thymus serum (Text-fig. 6). It is not possible to secure from the small lymph nodes of the rat sufficiently large suspensions to make absorption tests satisfactory. The experiment as it stands, however, seems strongly to point to a close biological relationship between the thymus cells and lymphocytes from other sources.



TEXT-FIG. 6. Experiment 9. Comparison of the action of thymotoxic serum upon thymus cells and lymph gland lymphocytes.

DISCUSSION AND SUMMARY.

The work of previous investigators gives the impression that it is easy to produce sera which both *in vitro* and upon injection are leukotoxic. At the same time the specificity of these leukotoxic sera for the particular type of cell used as antigen, and even for leukocytes in general, has been doubtful. The methods used have made certain possible factors of error unavoidable. Even careful washing of an organ or suspension cannot render it wholly blood-free, so that it is not surprising that the sera should be moderately hemolytic and hemagglutinative. Pearce[10] has shown that the injection of very

[10] Pearce, R. M., Concerning the specificity of the somatogenic cytotoxins, *J. Med. Research*, 1904, xii, 1.

small amounts of blood is sufficient to evoke the production of immune hemolysins. When such sera are injected the lesions, as Pearce states, may be due in part to the production of hemagglutinative thrombi, although this hardly seems to apply to the changes in lymphoid tissue described by Flexner. On the other hand, the lymphotoxic effect of hemolytic sera may be due to the lymphocytes injected with the red cells.

Our own experiments indicate that the lymphotoxic and agglutinative factors are to a considerable degree distinct from the hemolytic and hemagglutinative ones, since they can be separated from one another by absorption.

Further evidence is presented that the small thymus cells are biologically related to, if not identical with the lymphocytes derived from lymph glands.

I wish to acknowledge the assistance of Miss Kate Brogan in the technical part of the work.

EXPLANATION OF PLATE 13.

FIG. 1. Rat A. Thymus 24 hours after the injection of immune serum and complement.

FIG. 2. Rat B. Thymus 24 hours after the injection of normal serum and complement.

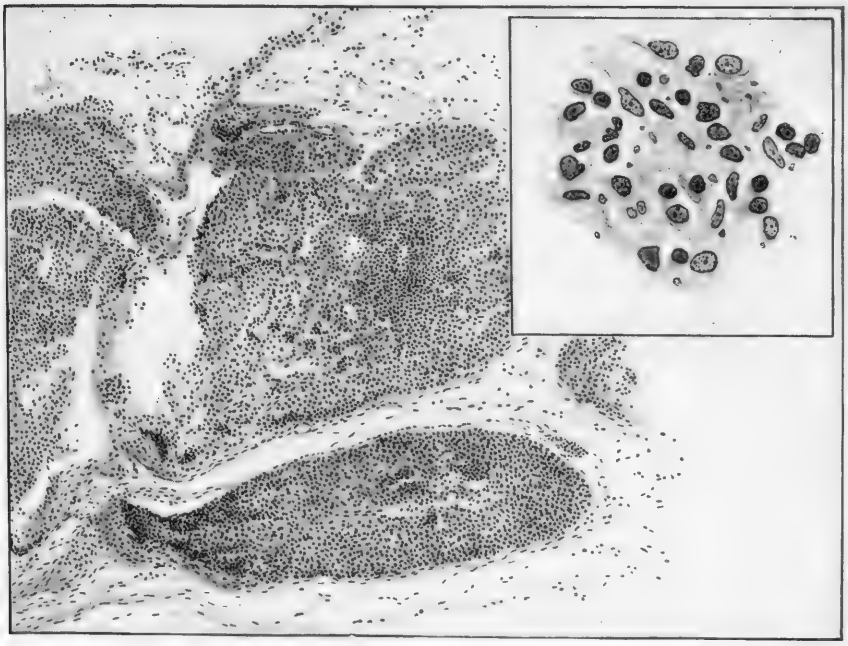


FIG. 1.

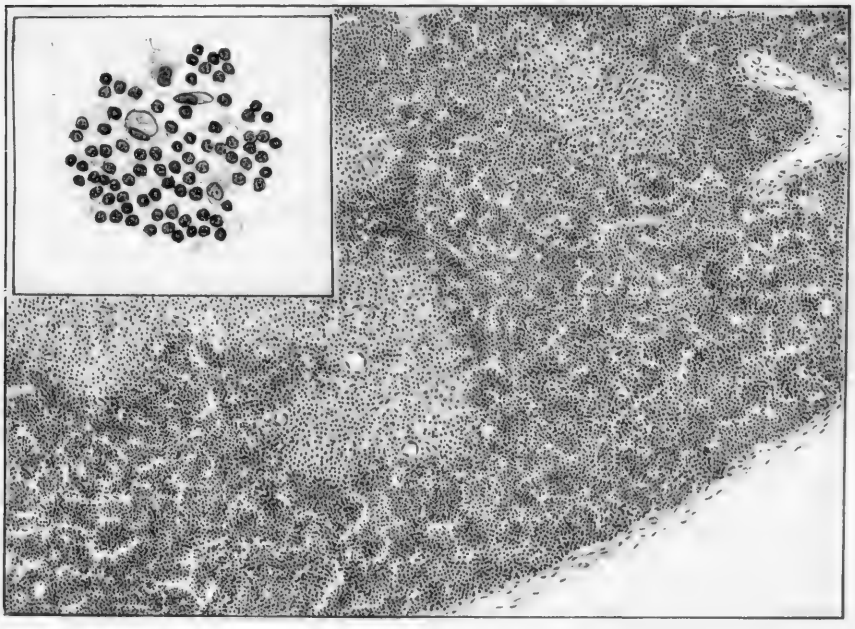


FIG. 2.

(Pappenheimer: Experimental studies upon lymphocytes. II.)

THE MECHANISM OF UREA RETENTION IN NEPHRITIS.

By FRANKLIN C. McLEAN, M.D.

(*From the Hospital of The Rockefeller Institute for Medical Research.*)

(Received for publication, March 3, 1917.)

The present paper furnishes additional evidence in support of the view of the mechanism of urea retention in Bright's disease advanced by Widal and Javal in 1904 (1). Upon their conception of this mechanism was based the later work of Ambard and Weill (2) and of the author (3). On account of a present general misconception of the mechanism of urea retention, it seems advisable to add further evidence in favor of the hypothesis originated by the French school, although all credit for the conception must be given to these writers. It is not proposed at this time to discuss the respective merits of diagnostic tests of renal function, either in regard to their reliability or simplicity. These studies have been carried on with the view of establishing certain laws which have biological significance and are apparently valid.

For the purposes of the present paper the term urea retention is intended to mean a relatively increased concentration of urea in the blood and tissues, such as may occur over rather long periods of time in acute and chronic nephritis and is attributed to a disturbance in renal function.

It does not refer to the more rapid accumulation of urea which may occur as a result of partial or complete suppression of urine, due not to a specific defect in the function which has to do with the excretion of urea, but to a suspension of that function which has to do with the elimination of fluid through the kidneys. Such accumulation commonly occurs in oliguria due to passive congestion of the kidneys, or to any other cause. Neither does it refer to the long continued positive nitrogen balance which has been described by some authors.

Widal and Javal (1) kept a woman aged 34 years under observation for 4 months, during which time the nitrogen balance was carefully observed. The patient

had been ill for 1 year with chronic nephritis and the general condition remained unchanged during the period of observation. The urine albumin varied between 1.0 and 2.5 gm. per liter. The chloride balance was perfect. They found that the patient came into nitrogen equilibrium readily after changes in the nitrogen intake, though somewhat more slowly than a normal individual. They also found that the concentration of urea in the blood bore a direct relation to the diet, and on a constant nitrogen intake was practically constant. After changes in diet the blood urea could be restored to the former level by a return to the original diet. They succeeded in causing the concentration of urea in the blood to increase from 0.36 to 1.93 gm. per liter by increasing the nitrogen intake. As a result of their experiment, they concluded that the fluctuations in the level of blood urea were due to the changes in diet, and that the increased concentration of urea in the blood, which occurred in response to increased nitrogen intake, effected the elimination of increased amounts of urea through the kidneys. The organism therefore remained in nitrogen equilibrium. In other words, high nitrogen intake, relatively high blood urea, and high rate of excretion were found to be parallel and interdependent phenomena, as were low nitrogen intake, relatively low blood urea, and low nitrogen output. The term relative is used since, as has been repeatedly pointed out, there can be no absolute standard for the concentration of urea in the blood, either in health or in disease. The above hypothesis received further support from the work of Ambard and Weill (2), who demonstrated that the rate of urea excretion depends primarily on the concentration in the blood. Numerous authors have confirmed the observation of Widal and Javal on the dependence of the concentration of urea in the blood on the level of protein intake.

The present paper deals chiefly with the detailed study of two cases similar to the study made by Widal and Javal, with a simultaneous study of the state of the urea excretion function by the index of urea excretion, based on Ambard's laws, previously described (3). These two cases reveal the interrelationship between nitrogen intake, blood urea concentration, and nitrogen output, and show that changes in the separate factors may occur while no change in the actual ability of the kidneys to excrete urea is demonstrable. Additional data are presented, from other cases, which show in common a characteristic not previously described.

Methods of Observation.

The two patients first described were cases of chronic nephritis selected on account of their diminished urea-eliminating function, with little or no impairment of the function of water excretion. They

were ambulatory patients, apparently not in a progressing state of the disease, and were able to tolerate the dietary changes well. On account of the length of the experiments it was thought advisable to allow a somewhat more varied diet than is usual in metabolism experiments, but the protein-containing foods were analyzed frequently, and were not varied from day to day. Protein was given chiefly in the form of eggs, bread, and milk. The chloride and fluid intake were also kept constant. Urine and stools were collected for 24 hour periods and analyzed daily for nitrogen. Blood urea was determined about twice a week, with a simultaneous urine analysis on a carefully timed specimen, in order to compute the index of urea excretion.[1]

Both the patients tolerated the regimen well and showed little or no change in the general condition at the end of the experiment.

Case 1.—C. P., male; age 48 years (Table I, Text-fig. 1).

Diagnosis.—General arteriosclerosis, chronic interstitial nephritis.

The patient was first admitted to the hospital on September 14, 1915, and was kept under observation until June 5, 1916. He complained of general weakness and loss of weight which had been progressing for 4 or 5 years. Physical examination revealed an advanced grade of peripheral arteriosclerosis. The heart was slightly enlarged. The rate and rhythm were normal. There were no murmurs. Fluoroscopic examination and the x-ray plate of the chest showed an enlarged aortic arch. The blood pressure was 180 systolic and 110 diastolic. The urine was clear, straw-colored, neutral; specific gravity 1,011. There was a heavy trace of albumin, but no sugar. Hyaline and finely granular casts were present, but not in large numbers. No red blood cells were found. The total elimination of phenolsulfonephthalein was 30 per cent in 2 hours on September 15.

The patient reentered the hospital in October and the nitrogen balance was determined from October 26 to December 29. The findings during this period

---

[1] The index of urea excretion is computed from the concentration of urea in the blood, the rate of urea excretion, the concentration of urea in the urine, and the weight of the patient, according to the following formula.

$$\text{Index of urea excretion (I)} = \frac{D\sqrt{C} \times 8.96}{Wt. \times Ur.^2}$$

D = gm. of urea excreted per 24 hours.
C = " " " per liter of urine.
Ur. = " " " " " " blood.
Wt. = body weight of individual, in kilos.

For further details consult the description of the index in previous papers (3).

TABLE I.

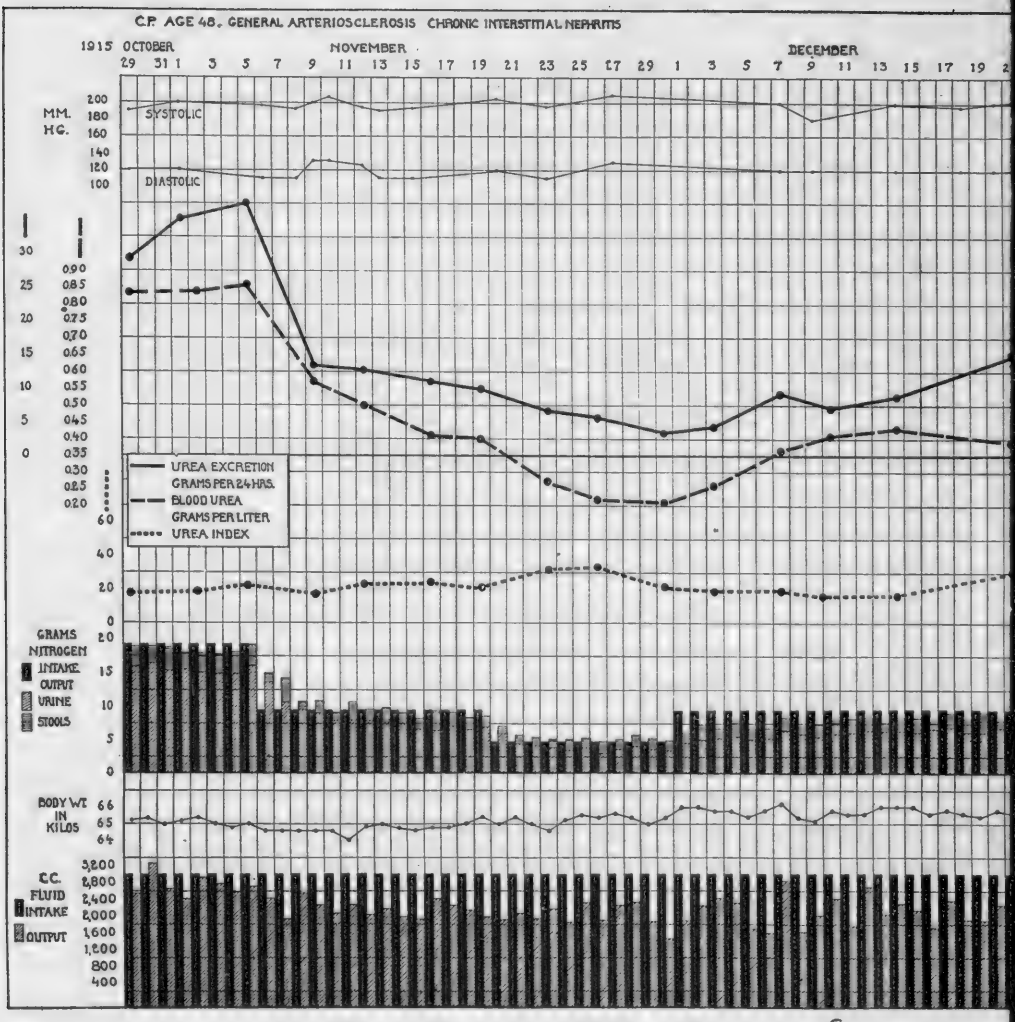
Case 1.

Date.	Weight.	Diet per 24 hrs.				Urine per 24 hrs.			Daily nitrogen balance.							Urea.		Blood pressure.	
		Protein.	Calories.	Sodium chloride.	Fluid.	Sp. gr.	Albumin per liter (Esbach).	Quantity.	Urea N per liter of urine.	Total N per liter of urine.	Total urine N in 24 hrs.	Stool N in 24 hrs.	Total N.			Urea per liter of blood.	Index of excretion (I).	Systolic.	Diastolic.
													Output in 24 hrs.	Intake in 24 hrs.	Balance.				
1915	*kg.*	*gm.*		*gm.*	*cc.*		*gm.*	*cc.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*		*gm.*			
Oct. 26	66.4	120.0	2,501	10.0	3,000	1,009	0.3	3,537	4.12	4.52	16.0	1.6	17.6	19.2	+1.6	0.825	18.4	190	120
" 27	65.2	120.0	2,501	10.0	3,000	1,010	0.2	2,719	4.25	5.30	14.4	2.0	16.4	19.2	+2.8	—	—	—	—
" 28	66.0	120.0	2,495	10.0	3,000	—	—	3,145	—	—	Lost.	3.0	—	19.2	—	—	—	—	—
" 29	65.2	120.0	2,495	10.0	3,000	1,010	0.25	2,540	5.18	6.25	15.9	3.2	19.1	19.2	+0.1	0.832	17.8	190	120
" 30	65.4	120.0	2,495	10.0	3,000	1,010	Tr.	3,265	4.32	5.04	16.5	2.5	19.0	19.2	+0.2	—	—	—	—
" 31	65.0	120.0	2,506	10.0	3,000	1,010	0.3	2,650	4.96	5.82	15.4	3.3	18.7	19.2	+0.5	—	—	—	—
Nov. 1	65.2	120.0	2,506	10.0	3,000	1,013	0.3	2,420	5.96	6.42	15.5	2.4	17.9	19.2	+1.3	—	—	200	120
" 2	65.4	120.0	2,506	10.0	3,000	1,012	0.3	2,930	4.45	5.15	15.1	2.7	17.8	19.2	+1.4	0.835	19.4	—	—
" 3	65.0	120.0	2,506	10.0	3,000	1,013	0.3	2,773	4.91	5.39	14.9	2.8	17.7	19.2	+1.5	—	—	—	—
" 4	64.8	120.0	2,506	10.0	3,000	1,010	0.25	2,568	4.81	5.79	14.9	3.1	18.0	19.2	+1.2	—	—	—	—
" 5	65.0	120.0	2,509	10.0	3,000	1,011	0.25	2,691	5.44	6.00	16.1	3.1	19.2	19.2	0	0.858	22.0	—	—
" 6	64.6	60.0	2,497	10.0	3,000	1,010	—	2,435	4.12	5.69	13.8	1.1	14.9	9.6	−5.3	—	—	197	110
" 7	64.6	60.0	2,497	10.0	3,000	1,010	0.25	1,931	4.64	5.52	10.7	3.5	14.2	9.6	−4.6	—	—	—	—
" 8	64.6	60.0	2,493	10.0	3,000	1,010	Tr.	2,553	2.99	3.78	9.7	1.0	10.7	9.6	−1.1	—	—	192	110
" 9	64.6	60.0	2,497	10.0	3,000	1,008	0.2	2,270	3.38	4.00	9.1	1.8	10.9	9.6	−1.3	0.569	17.4	200	130
" 10	64.6	60.0	2,497	10.0	3,000	1,007	0.1	2,073	3.16	3.88	8.0	1.1	9.1	9.6	+0.5	—	—	206	130
" 11	64.0	60.0	2,497	10.0	3,000	1,005	0.2	2,272	2.63	3.54	8.0	2.7	10.7	9.6	−1.1	—	—	—	—
" 12	64.8	60.0	2,493	10.0	3,000	1,008	0.2	2,026	3.41	3.91	7.9	1.6	9.5	9.6	+0.1	0.499	23.2	195	125
" 13	65.0	60.0	2,493	10.0	3,000	1,008	0.2	2,171	3.05	3.57	7.7	2.1	9.8	9.6	−0.2	—	—	190	110
" 14	64.8	60.0	2,493	10.0	3,000	1,009	0.25	1,988	3.42	3.82	7.6	1.7	9.3	9.6	+0.3	—	—	—	—

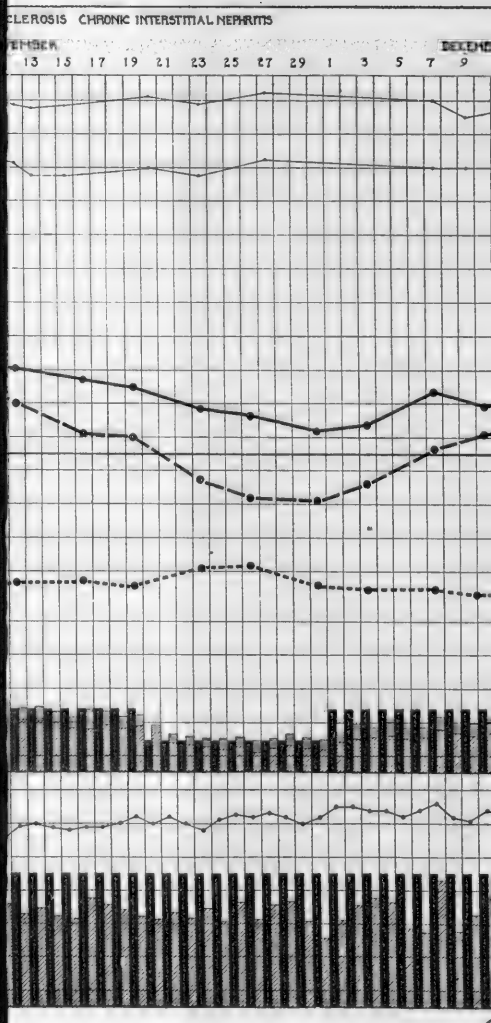
Nov. 15	64.6	60.0	2,497	10.0	3,000	1,007	0.1	1,918	2.75	3.50	6.7	1.5	8.2	9.6	+1.4	—	—	194	110
" 16	64.8	60.0	2,411	10.0	3,000	1,007	0.2	2,426	1.99	2.72	6.6	2.9	9.5	9.6	+0.1	0.411	24.0	—	—
" 17	64.8	60.0	2,411	10.0	3,000	1,006	0.25	2,251	2.66	3.19	7.2	1.9	9.1	9.6	+0.5	—	—	—	—
" 18	65.0	60.0	2,411	10.0	3,000	1,007	0.25	2,141	2.55	3.14	6.7	1.6	8.3	9.6	+1.3	—	—	—	—
" 19	65.4	60.0	2,411	10.0	3,000	—	Tr.	1,996	2.79	3.57	7.1	1.6	8.7	9.6	+0.9	0.402	21.0	—	—
" 20	65.0	30.0	2,798	10.0	3,000	1,006	0.2	1,915	2.26	3.15	6.0	1.1	7.1	4.8	−2.3	—	—	205	120
" 21	65.4	30.0	2,798	10.0	3,000	1,006	0.1	2,073	1.71	2.19	4.5	1.3	5.8	4.8	−1.0	—	—	—	—
" 22	65.0	30.0	2,798	10.0	3,000	1,006	Tr.	1,936	1.50	2.24	4.3	1.2	5.5	4.8	−0.7	—	—	—	—
" 23	64.6	30.0	2,798	10.0	3,000	1,005	0.2	2,177	1.36	1.78	3.9	1.2	5.1	4.8	−0.3	0.269	32.0	195	110
" 24	65.2	30.0	2,798	10.0	3,000	1,008	H.Tr.	1,852	1.27	1.97	3.6	1.6	5.2	4.8	−0.4	—	—	—	—
" 25	65.6	30.0	2,800	10.0	3,000	1,007	0.1	2,330	0.98	1.76	4.1	1.3	5.4	4.8	−0.6	—	—	—	—
" 26	65.4	30.0	2,798	10.0	3,000	1,005	Tr.	1,902	1.16	1.61	3.1	1.7	4.8	4.8	0	0.220	33.5	—	—
" 27	65.6	30.0	2,798	10.0	3,000	—	—	2,244	1.21	1.68	3.7	1.3	5.0	4.8	−0.2	—	—	210	130
" 28	65.4	30.0	2,798	10.0	3,000	—	Tr.	2,348	1.33	1.80	4.2	1.6	5.8	4.8	−1.0	—	—	—	—
" 29	65.0	30.0	2,793	10.0	3,000	1,007	0.3	1,873	1.37	2.00	3.7	1.5	5.2	4.8	−0.4	—	—	—	—
" 30	65.4	30.0	2,793	10.0	3,000	1,010	0.2	1,458	1.56	2.39	3.5	1.4	4.9	4.8	−0.1	0.211	21.4	—	—
Dec. 1	66.0	30.0	2,411	10.0	3,000	1,007	Tr.	1,896	1.72	2.49	4.7	1.3	6.0	9.6	+3.6	—	—	—	—
" 2	66.0	60.0	2,411	10.0	3,000	1,007	F.Tr.	2,249	1.62	2.29	5.2	2.1	7.3	9.6	+2.3	—	—	—	—
" 3	65.8	60.0	2,411	10.0	3,000	1,007	0.2	2,419	1.58	2.18	5.3	1.3	6.6	9.6	+3.0	0.272	19.1	—	—
" 4	65.8	60.0	2,411	10.0	3,000	1,007	—	2,319	1.84	2.40	5.6	2.3	7.9	9.6	+1.7	—	—	—	—
" 5	65.4	60.0	2,105	10.0	3,000	1,008	—	1,716	2.21	2.98	5.1	1.4	6.5	9.6	+3.1	—	—	—	—
" 6	65.8	60.0	1,995	10.0	3,000	1,009	0.2	1,598	2.56	3.32	5.3	1.4	6.7	9.6	+2.9	—	—	—	—
" 7	66.2	60.0	1,995	10.0	3,000	1,005	0.2	2,860	1.63	2.24	6.4	1.8	8.2	9.6	+1.4	0.363	19.2	200	120
" 8	65.4	60.0	1,995	10.0	3,000	1,010	0.4	1,637	2.72	3.59	5.9	1.1	7.0	9.6	+2.6	—	—	—	—
" 9	65.2	60.0	1,995	10.0	3,000	1,005	—	2,024	2.26	2.73	5.5	1.7	7.2	9.6	+2.4	—	—	180	120
" 10	65.8	60.0	1,995	10.0	3,000	1,006	—	2,412	1.93	2.52	6.0	2.0	8.0	9.6	+1.6	0.411	16.5	—	—
" 11	65.6	60.0	1,995	10.0	3,000	—	0.1	1,765	2.89	3.52	6.2	1.9	8.1	9.6	+1.5	—	—	—	—
" 12	65.6	60.0	1,995	10.0	3,000	—	0.1	2,710	2.15	2.42	6.5	1.8	8.3	9.6	+1.3	—	—	—	—
" 13	66.0	60.0	1,995	10.0	3,000	1,007	Tr.	2,051	2.37	3.08	6.3	1.2	7.5	9.6	+2.1	—	—	—	—
" 14	66.0	60.0	1,995	10.0	3,000	—	H.Tr.	2,292	2.32	2.81	6.45	1.9	8.35	9.6	+1.25	0.430	16.5	200	120

TABLE I—*Concluded.*

Date.	Weight.	Diet per 24 hrs.				Urine per 24 hrs.			Daily nitrogen balance.							Urea.		Blood pressure.	
		Protein.	Calories.	Sodium chloride.	Fluid.	Sp. gr.	Albumin per liter (Esbach).	Quantity.	Urea N per liter of urine.	Total N per liter of urine.	Total urine N in 24 hrs.	Stool N in 24 hrs.	Total N.			Urea per liter of blood.	Index of excretion (I).	Systolic.	Diastolic.
													Output in 24 hrs.	Intake in 24 hrs.	Balance.				
1915	*kg.*	*gm.*		*gm.*	*cc.*		*gm.*	*cc.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*			
Dec. 15	66.0	60.0	1,995	10.0	3,000	1,008	—	2,145	2.31	2.86	6.2	2.2	8.4	9.6	+1.2	—	—	—	—
" 16	65.6	60.0	1,995	10.0	3,000	1,010	—	1,737	2.97	3.87	6.7	1.2	7.9	9.6	+1.7	—	—	—	—
" 17	65.8	59.9	1,998	10.0	3,000	1,007	—	2,381	2.16	2.89	6.9	2.0	8.9	9.6	+0.7	—	—	—	—
" 18	65.6	59.9	1,999	10.0	3,000	1,009	0.1	1,916	2.60	3.48	6.2	1.8	8.0	9.6	+1.6	—	—	195	120
" 19	65.4	59.9	1,989	10.0	3,000	1,007	0.1	1,886	2.49	3.30	6.2	2.8	9.0	9.6	+0.6	—	—	—	—
" 20	65.8	60.0	1,995	10.0	3,000	1,008	0.3	2,259	2.39	3.02	6.8	1.1	7.9	9.6	+1.7	—	—	200	120
" 21	65.6	60.0	1,995	10.0	3,000	1,007	0.3	2,219	2.18	2.78	6.2	2.0	8.2	9.6	+1.4	0.388	30.0	—	—
" 22	66.0	90.0	2,200	10.0	3,000	—	0.35	2,083	2.68	3.42	7.1	1.8	8.9	9.6	+0.7	—	—	—	—
" 23	65.8	90.0	2,200	10.0	3,000	1,006	—	2,016	2.44	3.06	6.2	2.4	8.6	9.6	+1.0	—	—	—	—
" 24	65.6	90.0	2,200	10.0	3,000	1,010	—	1,945	3.29	4.15	8.1	2.1	10.2	12.0	+1.8	0.506	15.0	—	—
" 25	—	90.0	2,190	10.0	3,000	1,009	0.4	2,146	3.26	3.99	8.6	1.7	10.3	12.0	+1.7	—	—	—	—
" 26	65.2	90.0	2,200	10.0	3,000	1,007	—	2,080	3.39	4.51	9.4	1.8	11.2	12.0	+0.8	—	—	—	—
" 27	65.8	90.0	2,200	10.0	3,000	1,010	0.1	2,469	3.13	3.66	9.0	2.6	11.6	12.0	+0.4	—	—	—	—
" 28	65.6	90.0	2,200	10.0	3,000	—	—	1,868	4.43	5.08	9.5	3.7	13.2	12.0	−1.2	—	—	—	—
" 29	65.2	90.0	2,200	10.0	3,000	—	0.3	1,897	4.27	5.10	9.6	0.6	10.2	12.0	+1.8	0.564	19.8	220	130



TEXT-FIG. 1. Case 1. Graphic presentation of the data in Table I



. 1. Case 1. Graphic presentation of the data in T

able I

of observation are given in Table I. The patient tolerated the strict dietary regimen well, and complained only of gastric distress at times.

At the time of discharge from the hospital, the patient had gained in weight and strength, but there had been no change in the urinary or blood findings.

Case 2.—W. F., male; age 17 years (Table II, Text-fig. 2).

Diagnosis.—Chronic interstitial nephritis, aortic and mitral stenosis.

Admitted to the hospital on March 27, 1916. The patient seemed quite well, but was brought to the hospital on account of albuminuria. This had been discovered 1 year before, after a brother aged 18 years had died suddenly of uremia. The patient had had three attacks of rheumatism, 6, 4, and 2 years ago. Following the last attack, he had heart failure, with edema of both legs, which had been relieved by administration of a drug which caused a marked diuresis. No history of scarlet fever, measles, or diphtheria was obtained.

On physical examination the patient appeared to be a normal healthy boy aged 17 years, well nourished, and with good color. The heart was enlarged, and there was a systolic murmur at the apex and a diastolic murmur at the base. There were no signs or symptoms of heart failure. The blood pressure was 140 systolic and 85 diastolic. The urine was clear, straw-colored, and acid; specific gravity 1,012. There was a heavy trace of albumin, and no sugar. The sediment contained hyaline casts, leukocytes, and occasional red blood cells.

The patient was kept under close observation, and the nitrogen balance determined from March 28 to June 11. During this time there was no change in the general condition. On May 31, 40 gm. of urea were added to the diet, on June 1, 40 gm., and on June 2, 20 gm. No effect was noticed except that the patient asked for an increase in the daily allowance of fluid. The patient stated that he felt no change in his condition on or after these days.

Since discharge from the hospital the general condition has remained unchanged. When last examined on November 22, 1916, the urine was amber and turbid; specific gravity 1,012. 1.2 gm. of albumin per liter of urine were found (Esbach). There were a few casts in the sediment. The blood urea concentration was 0.774 gm. per liter and the urea index was 28.2.

Since Cases 1 and 2 are exactly similar, they will be discussed together. Both show the essential features described by Widal and Javal in their case; that is, a close parallelism between nitrogen intake, concentration of urea in the blood, and nitrogen output. Changes in nitrogen intake were always quickly followed by a change in the concentration of urea in the blood and in nitrogen output, so that nitrogen balance was reestablished on the new level. In Case 2 extremely wide fluctuations in the concentration of urea in the blood were obtained, from a minimum of 0.262 gm. per liter to a maximum of 2.542 gm. It is significant that the upper limit was far above the

TABLE II.

Case 2.

Date.	Weight.	Diet per 24 hrs.				Urine per 24 hrs.			Daily nitrogen balance.							Urea.		Blood pressure.	
		Protein.	Calories.	Sodium chloride.	Fluid.	Sp. gr.	Albumin per liter (Esbach).	Quantity.	Urea N per liter of urine.	Total N per liter of urine.	Total urine N in 24 hrs.	Stool N in 24 hrs.	Total N.			Urea per liter of blood.	Index of excretion (I).	Systolic.	Diastolic.
													Output in 24 hrs.	Intake in 24 hrs.	Balance.				
1916	*kg.*	*gm.*		*gm.*	*cc.*		*gm.*	*cc.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*			
Mar. 28	46.2	60.0	2,502	5.0	2,000	1,015	1.3	963	6.68	8.04	7.75	0.89	8.64	9.32	+0.68	0.610	22.5	140	85
" 29	46.6	60.0	2,502	5.0	2,000	1,015	0.8	970	7.37	8.38	8.12	0.60	8.72	9.32	+0.60	—	—	140	90
" 30	46.4	60.0	2,502	5.0	2,000	1,016	1.0	925	6.65	7.97	7.38	1.39	8.77	9.32	+0.55	—	—	130	85
" 31	46.2	60.0	2,502	5.0	2,000	1,017	1.0	782	6.44	7.70	6.02	1.47	7.49	9.32	+1.83	0.524	35.0	150	90
Apr. 1	46.4	60.0	2,502	5.0	2,500	1,015	0.5	1,095	4.75	5.70	6.25	0.87	7.12	9.32	+2.20	—	—	140	90
" 2	47.2	60.0	2,502	5.0	2,500	1,011	0.2	1,415	4.34	4.74	6.71	0.13	6.84	9.32	+2.48	—	—	—	—
" 3	47.6	60.0	2,502	5.0	2,500	1,007	0.4	2,196	2.56	3.09	6.78	2.42	9.20	9.32	+0.12	—	—	150	95
" 4	47.0	60.0	2,502	5.0	2,500	1,009	0.3	1,589	3.21	3.78	6.01	1.14	7.15	9.32	+2.17	0.434	31.0	140	85
" 5	46.0	60.0	2,502	5.0	2,500	1,009	0.3	1,751	2.96	3.62	6.34	2.32	8.66	9.32	+0.66	—	—	135	80
" 6	47.0	60.0	2,502	5.0	2,500	1,009	0.5	1,731	2.69	3.33	5.76	—	5.76	9.32	+3.56	—	—	140	85
" 7	46.8	60.0	2,502	5.0	2,500	1,010	0.2	1,557	3.45	4.05	6.30	0.86	7.16	9.32	+2.16	0.474	23.0	135	90
" 8	47.2	60.0	2,502	5.0	2,500	1,009	0.3	1,750	3.13	3.65	6.39	2.36	8.75	9.32	+0.57	—	—	125	70
" 9	47.0	60.0	2,502	5.0	2,500	1,009	0.3	1,656	3.36	3.89	6.45	0.62	7.07	9.32	+2.25	—	—	125	70
" 10	46.8	60.0	2,500	5.0	2,500	1,011	0.4	1,831	3.92	4.60	8.41	5.04	13.45	9.32	−4.13	0.587	23.0	140	90
" 11	47.0	60.0	2,500	5.0	2,500	1,010	0.2	1,505	3.74	4.40	6.66	0.98	7.64	9.32	+1.68	—	—	135	80
" 12	47.2	60.0	2,500	5.0	2,500	1,010	0.3	1,565	3.65	4.24	6.65	1.49	8.14	9.32	+1.18	—	—	135	80
" 13	47.0	60.0	2,500	5.0	2,500	1,012	0.25	1,300	3.98	5.02	6.53	1.50	8.03	9.32	+1.29	—	—	140	80
" 14	47.0	60.0	2,500	5.0	2,500	1,011	0.5	1,141	4.11	5.06	5.78	0.78	6.56	9.32	+2.76	0.559	26.8	135	90
" 15	47.4	60.0	2,500	5.0	2,500	1,013	0.35	1,050	4.48	5.64	5.93	2.24	8.17	9.32	+1.15	—	—	130	80
" 16	47.0	60.0	2,500	5.0	2,500	1,011	0.2	1,068	4.52	5.65	6.03	1.76	7.79	9.32	+1.53	—	—	135	80
" 17	47.0	60.0	2,500	5.0	2,500	1,012	0.4	1,331	3.82	4.91	6.54	1.91	8.45	9.32	+0.87	—	—	135	80

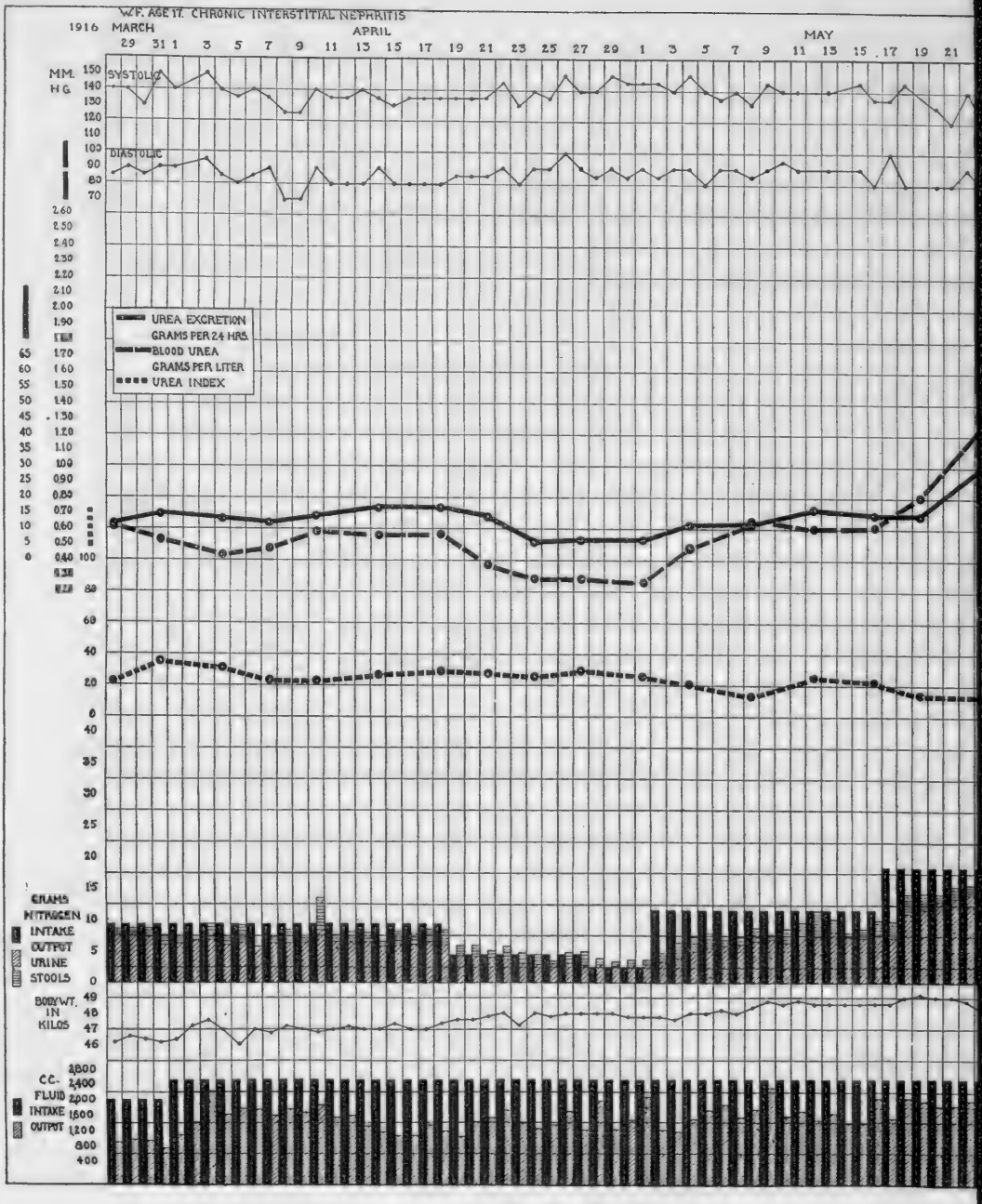
Apr. 18	47.4	60.0	2,500	5.0	2,500	1,012	0.25	1,274	3.79	4.91	6.12	2.42	8.54	9.32	+0.78	0.562	29.0	135	80
" 19	47.6	60.0	2,503	5.0	2,500	1,011	0.7	1,066	3.23	4.16	4.44	1.46	5.90	4.53	−1.37	—	—	135	85
" 20	47.6	60.0	2,503	5.0	2,500	1,009	0.2	1,435	2.28	2.97	4.25	1.76	6.01	4.53	−1.48	—	—	135	85
" 21	47.8	60.0	2,503	5.0	2,500	1,008	0.2	1,537	2.04	2.63	4.04	1.10	5.14	4.53	−0.61	0.377	28.0	135	85
" 22	48.0	60.0	2,503	5.0	2,500	1,008	0.3	1,720	1.68	2.20	3.78	2.09	5.87	4.53	−1.34	—	—	145	90
" 23	47.2	60.0	2,503	5.0	2,500	1,007	0.2	1,375	1.69	2.27	3.12	1.71	4.83	4.53	−0.30	—	—	130	80
" 24	48.0	30.0	2,503	5.0	2,500	1,010	0.2	1,243	1.62	2.37	2.95	1.55	4.50	4.53	+0.03	0.288	26.0	140	90
" 25	47.8	30.0	2,503	5.0	2,500	1,007	0.5	1,355	1.61	2.25	3.05	0.37	3.42	4.53	+1.11	—	—	135	90
" 26	48.0	30.0	2,503	5.0	2,500	1,007	0.3	1,684	1.32	1.87	3.15	1.68	4.83	4.53	−0.30	—	—	150	100
" 27	48.0	30.0	2,503	5.0	2,500	1,006	0.2	1,220	1.66	2.26	2.76	2.16	4.92	4.53	−0.39	0.287	29.5	140	90
" 28	48.0	15.0	2,501	5.0	2,500	1,007	0.3	1,941	1.06	1.47	2.85	0.98	3.83	2.40	−1.43	—	—	140	85
" 29	48.0	15.0	2,501	5.0	2,500	1,008	0.35	1,225	1.52	2.16	2.64	0.69	3.33	2.40	−0.93	—	—	150	90
" 30	47.8	15.0	2,501	5.0	2,500	1,006	0.3	1,470	1.20	1.66	2.44	1.15	3.59	2.40	−1.19	—	—	145	85
May 1	47.8	15.0	2,501	5.0	2,500	1,007	0.25	2,056	1.04	1.42	2.92	0.63	3.55	2.40	−1.15	0.262	26.0	145	90
" 2	47.8	75.0	2,499	5.0	2,500	1,011	0.4	1,216	1.90	2.75	3.34	1.38	4.72	11.5	+6.78	—	—	145	85
" 3	47.6	75.0	2,499	5.0	2,500	1,011	0.5	1,146	2.68	3.43	3.93	2.48	6.41	11.5	+5.09	—	—	140	90
" 4	48.0	75.0	2,499	5.0	2,500	1,009	0.3	1,480	2.72	3.38	5.00	2.38	7.38	11.5	+4.12	0.485	21.0	150	90
" 5	48.0	75.0	2,499	5.0	2,500	1,008	0.2	1,717	2.77	3.31	5.68	2.19	7.87	11.5	+3.63	—	—	140	80
" 6	48.2	75.0	2,499	5.0	2,500	1,009	0.2	1,858	2.60	3.17	5.90	0.90	6.80	11.5	+4.70	—	—	135	90
" 7	48.0	75.0	2,499	5.0	2,500	1,009	0.3	1,540	3.23	4.00	6.15	1.10	7.25	11.5	+4.25	—	—	140	90
" 8	48.4	75.0	2,499	5.0	2,500	1,010	0.25	1,751	3.11	3.82	6.69	2.06	8.75	11.5	+2.75	0.656	13.3	132	85
" 9	48.8	75.0	2,499	5.0	2,500	1,010	0.4	2,286	2.34	2.99	6.85	0.71	7.56	11.5	+3.94	—	—	145	90
" 10	48.6	75.0	2,499	5.0	2,500	1,008	0.4	1,557	3.44	4.16	6.46	2.17	8.63	11.5	+2.87	—	—	140	95
" 11	48.8	75.0	2,499	5.0	2,500	1,007	0.5	1,686	3.66	4.47	7.54	2.01	9.55	11.5	+1.95	—	—	140	90
" 12	48.6	75.0	2,499	5.0	2,500	1,010	0.5	1,483	4.18	4.97	7.39	4.04	11.43	11.5	+0.07	0.603	25.5	—	—
" 13	48.6	75.0	2,499	5.0	2,500	1,010	0.45	1,634	4.29	4.98	8.15	1.91	10.06	11.5	+1.44	—	—	140	90
" 14	48.6	75.0	2,499	5.0	2,500	1,009	0.5	1,377	4.44	5.34	7.35	0.62	7.97	11.5	+3.53	—	—	—	—
" 15	48.6	75.0	2,499	5.0	2,500	1,008	0.5	1,378	4.26	5.16	7.11	1.48	8.59	11.5	+2.91	—	—	145	90
" 16	48.6	75.0	2,499	5.0	2,500	1,007	0.45	1,988	3.26	3.84	7.63	2.24	9.87	11.5	+1.63	0.611	22.5	135	80
" 17	48.6	120.0	1,782	5.0	2,500	1,008	0.45	1,498	4.08	4.81	7.20	2.56	9.76	18.4	+8.64	—	—	135	100
" 18	49.0	120.0	1,782	5.0	2,500	1,009	0.6	2,024	4.66	5.50	11.13	3.00	14.13	18.4	+4.27	—	—	145	80

TABLE II—*Concluded.*

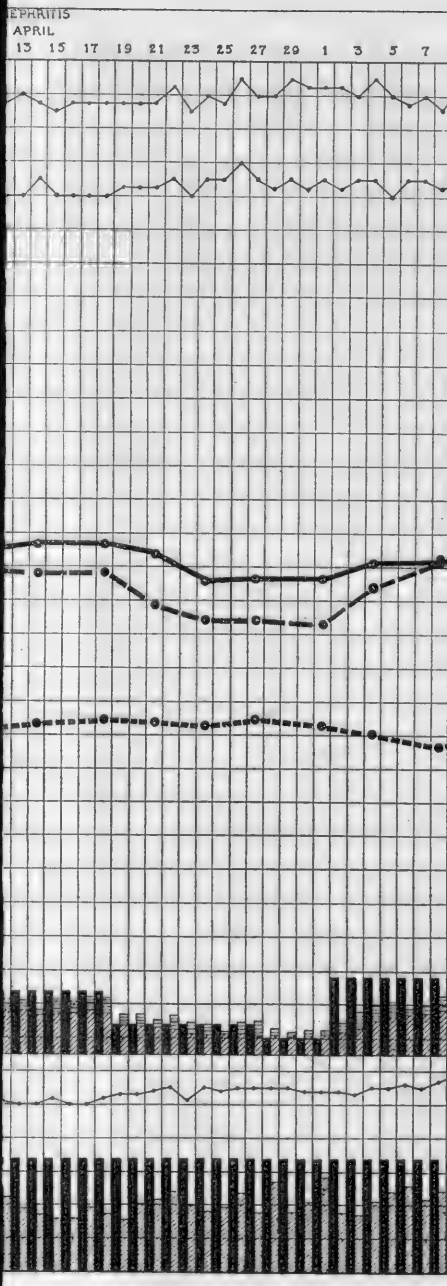
Date.	Weight.	Diet per 24 hrs.				Urine per 24 hrs.			Daily nitrogen balance.							Urea.		Blood pressure.	
		Protein.	Calories.	Sodium chloride.	Fluid.	Sp. gr.	Albumin per liter (Esbach.)	Quantity.	Urea N per liter of urine.	Total N per liter of urine.	Total urine N in 24 hrs.	Stool N in 24 hrs.	Total N. Output in 24 hrs.	Total N. Intake in 24 hrs.	Total N. Balance.	Urea per liter of blood.	Index of excretion (I).	Systolic.	Diastolic.
1916	*kg.*	*gm.*		*gm.*	*cc.*		*gm.*	*cc.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*			
May 19	49.2	120.0	1,782	5.0	2,500	1,010	0.5	1,921	5.24	6.08	11.70	2.56	14.26	18.4	+4.14	0.808	14.3	—	—
" 20	49.0	120.0	1,502	5.0	2,500	1,012	0.5	1,802	6.38	7.23	13.02	1.14	14.16	18.4	+4.24	—	—	130	80
" 21	49.0	120.0	1,502	5.0	2,500	1,010	0.5	1,826	6.65	7.39	13.50	1.82	15.32	18.4	+3.08	—	—	120	80
" 22	48.8	120.0	1,502	5.0	2,500	1,010	0.3	1,937	6.45	7.22	14.00	1.60	15.6	18.4	+2.8	—	—	140	90
" 23	48.2	120.0	1,502	5.0	2,500	1,011	0.3	1,775	6.92	7.62	13.52	3.66	17.18	18.4	+1.22	1.263	13.5	125	80
" 24	48.4	120.0	1,502	5.0	2,500	1,010	0.3	1,903	7.26	8.03	15.3	—	15.3	18.4	+3.10	—	—	140	90
" 25	49.0	120.0	1,502	5.0	2,500	1,011	0.5	1,778	7.75	8.44	15.0	1.68	16.68	18.4	+1.72	—	—	120	70
" 26	48.2	120.0	1,502	5.0	2,500	1,011	0.2	1,992	7.65	8.52	17.0	2.52	19.52	18.4	−1.12	1.314	18.1	140	90
" 27	48.0	120.0	1,502	5.0	2,500	1,014	0.2	1,930	8.00	8.66	16.70	1.95	18.65	18.4	−0.25	—	—	128	72
" 28	48.0	120.0	1,502	5.0	2,500	1,012	0.4	1,582	8.21	9.05	14.32	—	14.32	18.4	+4.08	—	—	—	—
" 29	47.6	120.0	1,502	5.0	2,500	1,010	0.3	2,420	6.46	7.04	17.02	0.73	17.75	18.4	+0.65	—	—	125	80
" 30	48.0	120.0	1,502	5.0	2,500	1,011	0.45	1,753	7.75	8.49	14.90	2.38	17.28	18.4	+1.12	1.192	18.8	125	80
" 31	48.0	120.0*	1,502	5.0	2,500	1,011	0.2	2,055	8.26	8.89	18.25	1.45	19.70	36.8	+17.1	—	—	120	75
June 1	47.8	120.0*	1,502	5.0	2,500	1,009	0.2	2,515	9.68	10.70	26.9	3.36	30.26	36.8	+6.54	—	—	125	80
" 2	47.0	120.0†	1,502	5.0	2,500	1,011	0.3	2,558	10.21	11.31	28.9	1.87	30.77	27.6	−3.17	2.542	8.3	120	75
" 3	46.6	60.0	2,502	5.0	2,800	1,010	0.3	1,548	9.65	10.40	16.1	1.32	17.42	9.32	−8.10	—	—	125	80
" 4	47.4	60.0	2,502	5.0	2,800	1,010	0.4	1,586	7.32	8.12	12.85	1.36	14.2	9.32	−4.88	—	—	—	—
" 5	48.0	60.0	2,502	5.0	2,800	1,008	0.5	1,812	4.27	5.18	9.40	2.04	11.44	9.32	−2.12	0.903	14.5	130	75
" 6	48.0	60.0	2,502	5.0	2,800	1,007	0.3	2,066	3.27	5.37	10.92	1.46	12.38	9.32	−3.06	—	—	130	75
" 7	48.2	60.0	2,502	5.0	2,800	1,006	0.3	1,647	2.66	4.75	7.82	4.3	12.12	9.32	−2.80	—	—	—	—
" 8	48.4	60.0	2,502	5.0	2,800	1,006	0.3	2,296	2.15	4.05	9.30	2.98	12.28	9.32	−2.96	0.470	22.2	130	85
" 9	48.6	60.0	2,502	5.0	2,800	1,007	0.2	2,142	2.34	4.07	8.72	1.44	10.16	9.32	−0.84	—	—	135	80
" 10	48.6	60.0	2,502	5.0	2,800	1,006	0.3	2,218	2.22	4.07	9.02	2.49	11.51	9.32	−2.19	—	—	130	80
" 11	48.6	60.0	2,502	5.0	2,800	1,006	0.2	2,135	2.18	4.08	8.72	1.79	10.51	9.32	−1.19	0.455	19.6	135	75

*40 gm. of urea added to diet.

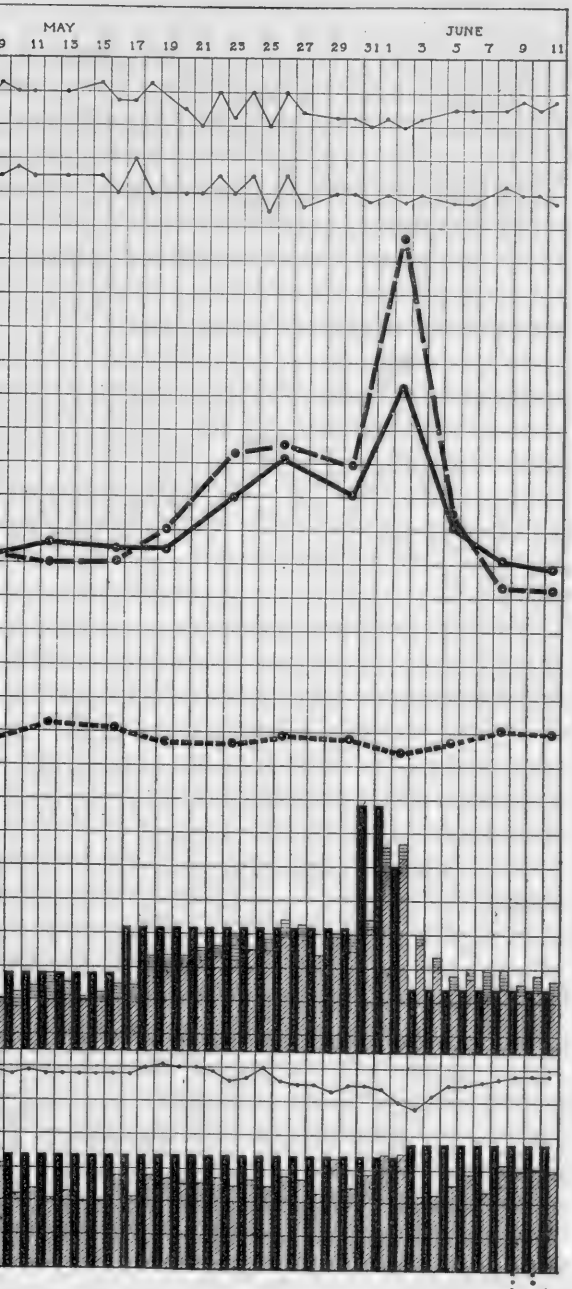
†20 gm. of urea added to diet.



TEXT-FIG. 2. Case 2. Graphic presentation of the data in Table II.



TEXT-FIG. 2. Case 2. Graphic presentation o



the data in Table II.

concentration in the blood at which Hewlett, Gilbert, and Wickett (4) believe that toxic effects of urea appear. The subject in this case is one in whom uremic symptoms should appear when the blood urea rises to a high level, if these symptoms are in any way referable to urea. As a matter of fact, no effect was noted beyond an increased fluid output, a slight loss in weight, and increased thirst.

The findings regarding urea excretion are of especial interest. In Case 1, with a variation in blood urea concentration from a minimum of 0.211 gm. per liter to a maximum of 0.858 gm., the index of urea excretion remained remarkably constant. In eighteen observations, with an average of 21.4, a figure below 16.4 is noted only once, and above 26.4 three times. The variations must be regarded as insignificant when the complexity of the formula and the possibility of error in obtaining the final result are taken into account.

In Case 2 a somewhat greater range of variation is noted. The most significant variation is that seen on June 2, when the blood urea reached 2.542 gm. per liter. Apparently the demand on the kidneys for the excretion of urea had exceeded their maximum capacity, and an actual accumulation had begun. This occurred, however, only when the nitrogen intake had been maintained at over 36 gm. per day for more than 2 days.

The two cases confirm the findings of Widal and Javal, and add the fact that no essential variation in the ability of the kidneys to respond to even high concentrations of urea in the blood at various levels of protein metabolism was demonstrable, until a very high level was reached; that is, the quantitative relationship that existed between the concentration of urea in the blood and the rate of its excretion remained the same at all levels of protein metabolism. The usual increase in blood urea concentration in nephritic individuals, on a liberal allowance of protein, can, therefore, hardly be attributed to diminished capacity for urea elimination. In these patients the capacity for urea excretion was far beyond the ordinary demands on that function, yet a relative increase in the blood urea concentration is evident at all levels of protein metabolism.

Several writers who have published observations obtained by means of Ambard's laws have emphasized the variations which occur in normal individuals. These variations, which may be quite wide,

are known to occur in the same individual and among different individuals. Attention has repeatedly been called to similar observations in previous papers (3). These variations, however, do not appear to affect the validity of Ambard's laws. Evidence that the laws are valid in as far as the variables considered are concerned seems conclusive. If this is true, then variations in the results must be accounted for by changes in the individual which introduce certain variable factors not represented in the formula. We have previously suggested that the variation may be due in part to such variable factors.

In the first place, it has been generally recognized that the results are more nearly constant in nephritic individuals than in normal individuals. The cases outlined above illustrate the constancy of the laws in two individuals with a marked disturbance in the elimination of urea. It is in normal individuals, as has already been noted, that a marked variability occurs in the index, although the findings do not, as a rule, give rise to doubt as to whether the individual falls within a normal or an abnormal group. Variability or elasticity of this sort probably represents a factor of safety. Its loss, therefore, would seem to be a sign of disease, even though this loss occurs before any quantitative diminution in renal function appears. If this assumption is correct, we should expect to find individuals in whom normal, or nearly normal quantitative estimations of renal function are found, but in whom, on repeated examination, there is the striking constancy in numerical results of the application of Ambard's laws that is shown by distinctly abnormal cases. These individuals would not, then, be considered normal. Cases 3 to 6 (Tables III to VI) show this constancy in numerical value of the index.

Case 3.—E. C., female; age 39 years (Table III).

Diagnosis.—Mitral stenosis, arterial hypertension, hypertrophy of heart.

The patient was under observation from December 2, 1915. She complained of headache, cough, and dyspnea on exertion, and presented the physical signs of mitral stenosis. There was no edema. Her blood pressure varied while under observation from 210 systolic and 130 diastolic, to 140 systolic and 80 diastolic after rest in bed. Examination of the urine on December 11 showed it to be clear, amber, acid, and to contain a very faint trace of albumin but no casts or

sugar; specific gravity 1,027. On repeated examination the amount of albumin varied from negative to a trace. Hyaline casts were found but once. The patient was subjected to alterations in diet, but the nitrogen balance was not determined. Her general condition improved with complete rest, but there was no change in the physical signs, except for lowering of blood pressure.

The total elimination of phenolsulfonephthalein on December 2 was 65 per cent in 2 hours.

The findings regarding urea excretion in the six observations which were made are tabulated in order, according to the concentration of urea found in the blood.

TABLE III.

Case 3.

Date.	Weight.	Urine in 24 hrs.	Urea.			Index of excretion (I*).
			Per liter of blood (Ur.*).	Per liter of urine (C*).	Amount in 24 hrs. (D*).	
1915-16	*kg.*	*cc.*	*gm.*	*gm.*	*gm.*	
Dec. 31.....	72.2	7,100	0.107	1.30	9.07	112
Jan. 26.....	69.6	9,300	0.170	1.69	15.7	91
Dec. 3.....	76.0	4,900	0.187	3.13	15.3	92
" 2.....	75.5	10,200	0.196	2.23	22.8	105
Jan. 11.....	72.0	2,020	0.252	8.82	17.85	104
Dec. 14.....	74.6	2,600	0.300	9.35	24.3	99

* For the explanation of these abbreviations see the formula for the index of urea excretion.[1]

Case 4.—J. F., male; age 43 years (Table IV).

Diagnosis.—Mitral stenosis, auricular fibrillation, chronic heart failure.

The patient was admitted on November 27, 1915, and remained in the hospital until July 11, 1916, when he died. During the entire stay he presented a typical picture of chronic cardiac valvular disease with heart failure. Response to treatment was at times striking, but never prolonged, and the patient was never entirely free from edema. The amount of albumin in the urine varied from a very faint trace, at times when the elimination of urine was best, to 3.0 gm. per liter (Esbach) at times when the other signs of cardiac failure, such as dyspnea, cyanosis, edema, and scanty urine, were worst. Hyaline and granular casts were present in abundance at times, but were few in number or absent when the urine albumin was low in amount. Red blood cells of undetermined origin were constantly present. The findings regarding urea excretion also varied with the condition of the patient, quantitatively normal findings being present at times when other signs were favorable. The table shows four successive observations made during one of these periods. At this time the patient was voiding an amount

of fluid in excess of his intake, felt fairly well, and the edema was disappearing. The urine on March 11, was amber, turbid, and there was a very faint trace of albumin; specific gravity 1,019. The sediment contained red blood cells, but no casts were seen.

TABLE IV.

Case 4.

Date.	Weight.	Urine in 24 hrs.	Urea.			Index of excretion (I).
			Per liter of blood (Ur.).	Per liter of urine (C).	Amount in 24 hrs. (D).	
1916	*kg.*	*cc.*	*gm.*	*gm.*	*gm.*	
Mar. 7.....	63.0	1,220	0.345	18.15	22.1	112
" 8.....	62.6	900	0.294	17.65	15.9	108
" 9.....	62.0	1,200	0.279	13.4	16.1	110
" 12.....	60.8	1,280	0.333	17.2	22.0	121

Case 5.—M. G., male; age 58 years (Table V).

Diagnosis.—General arteriosclerosis, chronic myocarditis, and hypertrophy of heart.

The patient was admitted to the hospital on December 8, 1915, and remained under observation until April 3, 1916. On admission he complained of swelling of the feet and dyspnea on exertion. There was marked edema of both lower extremities. The heart was considerably enlarged, the rate was rapid, and the rhythm normal. A systolic murmur was heard at the apex. The urine was turbid, dark amber, acid, and contained 0.25 gm. of albumin per liter (Esbach); specific gravity 1,013. There was no sugar. The sediment contained many leukocytes and leukocytic and hyaline casts. After a few days in bed without change in condition a marked diuresis was induced with theocin (0.9 gm. daily for 3 days). The edema of the extremities rapidly disappeared, there being practically none demonstrable on December 17, although fluid in the chest persisted for some time longer. At this time there was only a faint trace of albumin in the urine, with numerous leukocytes, but no casts in the sediment. The general condition of the patient remained good. The table shows all observations made on urea excretion from the time of admission until January 5, 1915, when theocin was again administered. The patient later had an attack of hemiplegia, and one of bronchopneumonia, but was discharged from the hospital in fairly good condition on March 5, 1916. He died on May 31, of a second attack of hemiplegia.

TABLE V.

Case 5.

Date.	Weight.	Urine in 24 hrs.	Urea.			Index of excretion (I).	Medication.
			Per liter of blood (Ur.).	Per liter of urine (C).	Amount in 24 hrs. (D).		
1915	*kg.*	*cc.*	*gm.*	*gm.*	*gm.*		
Dec. 9	91.6	1,060	0.403	19.43	20.6	55	
" 12	91.6	2,000	0.374	13.8	27.6	72	
" 13	91.0	1,535	0.381	14.1	21.7	55	
" 14	89.2	2,840	0.336	7.78	22.1	55	Theocin.
" 15	86.4	5,360	0.232	6.4	34.2	167	"
" 16	83.8	3,400	0.234	6.98	23.7	123	"
" 20	82.2	2,160	0.384	14.1	30.4	85	
" 23	80.2	2,360	0.361	12.1	28.6	85	
" 27	78.0	1,840	0.461	19.6	36.0	86	
" 30	78.0	1,620	0.440	20.7	36.6	89	
1916							
Jan. 3	78.6	2,000	0.457	19.85	39.7	97	
" 5	77.8	1,800	0.416	17.4	31.3	87	

Case 6.—J. A., male; age 43 years (Table VI).

Diagnosis.—Chronic interstitial nephritis, hypertrophy of heart.

The patient was first admitted to the hospital in March, 1915, and has been under almost constant observation since that time. He suffers from frequent attacks of cardiac insufficiency associated with severe edema, ascites, and hydrothorax. He responds readily to treatment, and generally leaves the hospital in fairly good condition, without dyspnea, cyanosis, or edema. Table VI records the observations made during the patient's third stay in the hospital, and all the findings of the period from March 23 to April 20 are shown. An unusual constancy in the index of excretion is shown. During this time the patient was improving rapidly while taking digipuratum, 0.2 gm., and diuretin, 0.2 gm. daily, 5 days out of 7. The urine was clear, amber, and acid; specific gravity 1,009. There was a trace of albumin, and a few hyaline casts were seen in the sediment. Fluid excretion during the period was constantly in excess of the intake. At other times the findings have shown a marked difference from those detailed below. The patient is at present again in the hospital, having been admitted for the fifth time.

TABLE VI.

Case 6.

Date.	Weight.	Urine in 24 hrs.	Urea.			Index of excretion (I).
			Per liter of blood (Ur.).	Per liter of urine (C).	Amount in 24 hrs. (D).	
1916	*kg.*	*cc.*	*gm.*	*gm.*	*gm.*	
Mar. 23.....	85.6	1,745	0.326	9.53	16.62	50
" 24.....	84.8	1,300	0.312	11.6	15.1	56
" 25.....	84.0	2,600	0.310	7.46	19.4	59
" 27.....	78.6	1,400	0.310	9.4	13.15	48
" 28.....	77.2	940	0.300	12.22	11.5	52
" 30.....	76.6	860	0.337	14.52	12.5	51
Apr. 1.....	76.0	1,100	0.340	11.95	13.15	46
" 3.....	76.6	1,200	0.407	16.4	19.65	56
" 4.....	76.8	1,200	0.423	17.25	20.7	56
" 6.....	74.8	1,100	0.399	15.55	17.1	51
" 8.....	72.0	700	0.354	18.15	12.7	54
" 10.....	71.8	1,015	0.447	18.8	19.1	52
" 13.....	72.2	1,300	0.507	19.2	24.95	52
" 16.....	72.0	940	0.411	16.35	15.4	46
" 18.....	71.0	900	0.414	17.8	16.05	50
" 20.....	69.8	800	0.329	15.8	12.6	59

Cases 3, 4, and 5 show results regarding urea excretion resembling the normal as far as numerical value is concerned, but differing from healthy individuals in that they show no tendency to variation in the numerical value of the index. The significance of such findings has not yet been determined, but they have a distinct bearing on the physiology of urea excretion. We assume that as a result of some unknown pathological change, a fixation of certain factors involved in urea excretion which are variable in normal individuals has occurred. The numerical results remain constant, even when wide variations in the level of protein metabolism are brought about by changes in the diet. This was shown to be true in Case 3. Case 6 shows findings more definitely pathological, but here too is a high degree of constancy in the index.

In Case 5, during a time when the circulation was obviously poor and the urine showed evidence of renal passive congestion, the index was nearly constant at 55. The administration of theocin was followed by marked diuresis and a temporary increase in the index.

The edema disappeared as well as the signs of passive congestion. The index then remained constant at about 85. The index was accordingly capable of variation, but not in the manner that variation ordinarily occurs in normal individuals. One must conclude that the factors which are usually variable in normal individuals were unusually fixed in this individual. Case 4 resembled Case 5.

Case 3 was one of those we are accustomed to include in the group of primary hypertension, in view of the absence of demonstrable renal lesion. According to the view just presented, the kidneys of this patient have lost their normal elasticity. Whether this loss represents the forerunner of an actual impairment of renal function must be left for further investigation to determine.

DISCUSSION.

The experimental facts presented demonstrate that the occurrence of a relatively increased concentration of urea in the blood follows the increased resistance with which diseased kidneys oppose the passage of urea. When, as the result of feeding an increased amount of nitrogen, the concentration of urea in the blood rises, a parallel increase in the rate of excretion of urea occurs. When a point is reached at which the rate of urea excretion is kept equal to the rate of formation by the organism, the level of urea in the blood ceases to rise and the organism remains in nitrogen equilibrium. But when the nitrogen intake in this individual is diminished, urea is excreted for a time more rapidly than it is formed, until the level of urea in the blood falls and reaches a point such that the rate of urea excretion is again equal to the rate of urea formation, and the organism is once more in nitrogen equilibrium. The experiments illustrate the events occurring in the so called retention of urea.

Fluctuation in the level of urea in the blood thus occurs in abnormal individuals in exactly the same way in which it occurs in normal individuals. The mechanism of excretion remains the same, but on account of the increased resistance with which the kidneys oppose the passage of urea the rate of urea excretion becomes relatively less rapid than in the case of normal individuals, and the level of urea in the blood becomes relatively higher. It is the rate of urea excretion, relative to the other conditions found and relative to the usual nor-

mal rate, that the index of urea excretion, based on Ambard's laws, expresses in numerical form.

As has been shown, the index of urea excretion may vary within wide limits in normal persons. These limits may be known as the limits of normality, and their extremes usually lie between 80 and 200. Fluctuations to this extent must, we think, be emphasized as well as the degree of constancy on which insistence has until now been placed, and which originally supplied the evidence on which the laws of Ambard were based.

In certain individuals, however, not fluctuation but extreme constancy, to which we now apply the term fixation, is the rule. Fixation is, in some cases, too striking to be considered as due to coincidence. It is dwelt upon in this paper on account of the support which it gives to the laws of Ambard. It appears now to be desirable to distinguish between extreme constancy or fixation and the relative constancy which we find in normal persons. The relative constancy of normal individuals is intended to include, as has been stated, a certain fluctuation as far as numerical values are concerned. It must be taken to include that degree of fluctuation, almost always above the level of 80, which normal people often exhibit. Fluctuation of this type has been found by all observers and is greater than the range of experimental error. The cause and the significance of fluctuation is difficult to determine. It may depend either upon the influence of variable factors not determined as yet, and therefore not included in the formula, or on the fact that the ascertainable values now included in the formula do not hold that relation to each other which the laws imply. The latter interpretation we hold to be inadmissible because there is a sufficient degree of constancy, even under conditions where fluctuation occurs, to furnish strong evidence of the validity of the laws. In certain abnormal conditions where fixation has been shown to occur, the striking degree of constancy obtained must be interpreted as adding greatly to this evidence already furnished by the degree of constancy obtained in normal individuals. The pathological significance of this fixation, especially when it occurs within the limits of normality, is not yet determined.

Finally, we have failed to note uremic symptoms in a patient in whom the concentration of urea in the blood rose, under experimental conditions, to 2.542 gm. per liter.

CONCLUSIONS.

1. Urea retention, in the sense of a relatively increased concentration in the blood, is the result of increased resistance to the excretion of urea through the kidneys.

2. The relatively increased concentration of urea in the blood overcomes the increased resistance to excretion, and the organism is thereby maintained in nitrogenous equilibrium.

3. The laws formulated by Ambard for the excretion of urea apply in the condition of urea retention under a widely varying range of conditions, as to nitrogen intake and excretion.

4. The numerical value of Ambard's constant changes in urea retention, but the relation of the variable factors to one another remains otherwise unchanged.

5. In certain individuals, with otherwise normal findings in regard to urea excretion, an unusual degree of constancy, to which we have applied the term fixation, has been found in the numerical results obtained by the application of Ambard's laws. These individuals are regarded, as the result of this study, as probably abnormal, but the pathological significance of the fixation has not been determined.

BIBLIOGRAPHY.

1. Widal, F., and Javal, A., *Compt. rend. Soc. biol.*, 1904, lvii, 301, 303.
2. Ambard, L., *Compt. rend. Soc. biol.*, 1910, lxix, 411, 506.
   Ambard, L., and Weill, A., *J. physiol. et path. gén.*, 1912, xiv, 753.
3. McLean, F. C., and Selling, L., *J. Biol. Chem.*, 1914, xix, 31.
   McLean, F. C., *J. Exp. Med.*, 1915, xxii, 212, 366.
   McLean, *J. Am. Med. Assn.*, 1916, lxvi, 415.
4. Hewlett, A. W., Gilbert, Q. O., and Wickett, A. D., *Arch. Int. Med.*, 1916, xviii, 636.

EXPERIMENTS ON THE CAUSATION AND AMELIORATION OF ADRENALIN PULMONARY EDEMA.

By JOHN AUER, M.D., and FREDERICK L. GATES, M.D.

(*From the Department of Physiology and Pharmacology of The Rockefeller Institute for Medical Research.*)

Plates 14 to 16.

(Received for publication, March 21, 1917.)

During an investigation of the absorption of drugs[1] by the pulmonary air passages of the rabbit we observed that an intratracheal injection of adrenalin, after a double vagotomy, apparently produced a fulminant pulmonary edema much more readily than when the vagi were intact. In the present paper we shall report a study of this subject, together with other series of experiments, which finally led to the recognition of a practically ignored respiratory factor in the production of adrenalin pulmonary edema.

That adrenalin may cause pulmonary edema in the normal rabbit was noted early in the experimental investigation of this substance. Bouchard and Claude[2] in 1902 observed pulmonary edema and death in the normal rabbit after the intravenous injection of adrenalin, an observation abundantly corroborated. Pulmonary edema in the rabbit may also be obtained when the adrenalin is injected subcutaneously, though tremendous doses are then necessary; thus Battelli and Taramasio[3] produced this effect with the subcutaneous injection of 10 mg. of adrenalin per kilo.

The facilitating action of vagus section upon the occurrence of adrenalin pulmonary edema in the rabbit has not to our knowledge been observed before. In artificially induced hydremic plethora, however, the vagi, according to Kraus,[4] play in this respect a decisive part.

[1] Auer, J., and Gates, F. L., *J. Exp. Med.*, 1916, xxiii, 757.

[2] Bouchard, C., and Claude, H., *Compt. rend. Acad.*, 1902, cxxv, 928.

[3] Battelli, F., and Taramasio, P., *Compt. rend. Soc. biol.*, 1902, liv, 815.

[4] Kraus, F., *Z. exp. Path. u. Therap.*, 1913, xiv, 402.

EXPERIMENTAL.

Methods.

Rabbits only were used in this investigation. The animals were placed on an electric warming pad and ether anesthesia was employed for operative procedures. A wide glass tube about 2 cm. long was tied into the trachea. When blood pressure was recorded the right carotid artery was connected to the mercury manometer by tubing filled with half saturated sodium sulfate. When the lungs and heart were inspected the sternum was split longitudinally; usually both pleural sacs were opened. Intratracheal insufflation was then started so that the lungs were always moderately distended. The vagi, when sectioned, were divided in the neck.

The adrenalin employed was the ordinary commercial product obtained in the market, and was slowly injected by syringe through the tracheal tube. The dose was usually 0.25 cc. per kilo of body weight; occasionally more or less was used.

When atropine was administered, this drug was usually injected intratracheally; in a few instances the atropine was injected intramuscularly.

As criteria for the presence of pulmonary edema we utilized the appearance of foam in the trachea and the autopsy findings. As a rule, the medulla was punctured in the survivors 30 minutes after the injection; in some instances 1 to 3 hours were allowed to elapse.

Although the appearance of the typical tracheal foam is positive proof of the existence of pulmonary edema, yet its absence by no means indicates the absence of pulmonary edema. Râles may be audible to the unaided ear and easily felt by the fingers and yet the trachea may remain perfectly clear of foam. This happens especially when the injected animals do not struggle; there is then no mechanical compression of the lungs and the foam and fluid remain in the lower air passages. For this reason we killed the surviving animals after a period of time by medullary puncture and at once examined the lungs.

Several series of experiments were carried out. In the first series both vagi were divided and after not less than 10 minutes the adrenalin was injected. The controls for this series were normal animals with intact vagi, but in many of them the adrenalin was injected repeatedly, thus rendering the control test especially severe.

In another series with divided vagi, the behavior of the heart was examined by inspection, the sternum being split lengthwise and the pericardial sac opened. Artificial respiration was instituted before the lungs were exposed. The adrenalin was always administered repeatedly. In a subsidiary series artificial respiration, with and

without ether, was given to rabbits with chests intact but with vagi divided.

In still another series the vagi were again divided, but atropine was administered before the injection of adrenalin.

In a final series the effect of tracheal stenosis upon the production of pulmonary edema in normal rabbits (vagi intact) was tested.

Results in Series with Divided Vagi.

The vagi were divided before the adrenalin was injected. The average dose was 0.25 cc. per kilo, and as a rule only one injection was given. The following protocol will give a clear picture of a typical experiment.

Rabbit 1.—Gray male; weight 1,740 gm.

10.45. On electric pad at medium.

10.58. Start ether.

11.02. Operation completed; both vagi divided, cannula in trachea. Stop ether.

11.08. Respiration rapid, easy. No inspiratory stoppage. Heart moderately rapid, regular, good strength.

11.10. Change pad to low.

+11.12. 0.52 cc. adrenalin into trachea (0.3 cc. per kilo); no loss.

11.13. Respiration more shallow and more rapid.

11.13½. Respiration slowed, deeper, with retraction of costal margin; respiration stopped.

11.14½. Respiration starts, slow, deep, with retraction of costal margins. Heart very rapid, apparently regular, good strength.

11.15. Struggle; pink foam in tracheal tube.

11.15¼. Pink foam pouring from tracheal tube.

11.16. Struggle, marked outflow of pink foam. Heart apparently regular, rapid (palpation).

11.17. Respiration rapid; strong retraction of costal margin.

11.18. Convulsive struggles; pink foam and fluid pouring from tracheal tube. No heart palpable. Occasional gasp. Pupils wide.

11.19. No respiration. No heart palpable.

Autopsy at once. No excess fluid in peritoneal cavity. Well marked peristalsis of small gut and cecum; cecum full of gas. Spleen and liver congested. Kidneys apparently normal. No hemorrhages in diaphragm. Lungs do not collapse. Heart large, fills entire pericardial sac. Auricles and ventricles dilated. Heart flabby, large, with no hemorrhages on pericardial or endocardial surfaces. No clot in pulmonary artery or aorta. Lung and trachea excised;

lungs large, erect, heavy, surfaces covered with many small hemorrhages, often confluent. Lower lobes show most hemorrhages; bluish black-red color; upper lobes pink. In trachea pink foam and white foam, alternately in layers. On section, lower lobes extremely juicy, like a wet sponge; not much air, chiefly red fluid with some foam. Upper lobes contain a large amount of white foam, not much fluid; not heavy like lower lobes (Fig. 2).

Of 27 rabbits whose vagi had been divided, 21 showed on autopsy a marked pulmonary edema of the general type described in the preceding protocol. 14 died within 20 minutes of the single intratracheal injection of adrenalin, and of these 10 died within 10 minutes, showing the general symptoms noted above. In only 6 did the autopsy show slight or no edema. Thus in about 78 per cent (21 out of 27) of the animals a single intratracheal injection of a moderate dose of adrenalin caused a marked pulmonary edema.

Control Series with Vagi Intact.

In this group of sixteen animals the adrenalin was usually injected repeatedly into the trachea, the time interval varying between 4 to 20 minutes. The dosage was usually 0.25 cc. per kilo. In addition to the intratracheal injection a number of the rabbits received intramuscular injections of adrenalin.[5] This series therefore forms a severe control for the group with divided vagi.

The results are briefly as follows: None of the rabbits, in spite of the repeated injections, died within 20 minutes; 3 succumbed after 40 to 45 minutes. The survivors (13) were either killed by medullary puncture 15 to 60 minutes after the first dose of adrenalin or were etherized after 15 minutes and the heart was clamped off. Autopsy revealed in no instance a pulmonary edema of the degree which was found in the series with divided vagi. Pulmonary edema, however, was present to some degree in the lungs of most of the animals; in 6 rabbits the edema was moderate, in the remaining 6 it was slight; one autopsy record was lost.

A comparison of the two series shows clearly that the intratracheal injection of adrenalin exerts a much severer effect in vagotomized

[5] Specimen protocols in tabular form will be found in our previous article, Auer and Gates,[1] pp. 760–761.

than in normal animals. Thus 14 out of 21 vagotomized animals died acutely in 20 minutes after a single intratracheal injection of about 0.25 cc. of adrenalin per kilo, while only 3 out of 16 normal animals succumbed within 40 minutes, although the normal animals received the adrenalin repeatedly. In addition it must be emphasized that the degree of pulmonary edema was much greater in the vagotomized series than in the control series whose vagi were intact.

Behavior of the Heart.—In a number of experiments where the blood pressure was recorded, it was observed repeatedly that the blood pressure at one time or another after the adrenalin injection showed sudden, profound drops varying in duration from 10 seconds to 3 minutes associated usually with convulsions after the low pressure level had been attained. Foam in the trachea was noted in some cases, especially those in which the low pressure level was maintained for some time. In Fig. 1, for example, foam first appeared in the trachea after the second series of convulsions following the second abrupt drop of blood pressure.

These abrupt drops of blood pressure were obtained not only in animals with intact vagi, but also in those whose vagi had been sectioned. The cause of these drops was therefore peripheral and probably located in the heart itself. In order to gain information on this point the heart was exposed in a series of seven etherized animals by splitting the sternum and opening the pericardial sac. Usually both pleural sacs were also opened, the respiration being maintained by intratracheal insufflation. In all except one animal the vagi were severed. A specimen protocol will illustrate the observations made and their time relations.

Rabbit 2.—Gray female; weight 1,885 gm.

10.20. On pad at low. Ether.

10.46. Operation finished: both vagi divided; cannula in trachea; chest split longitudinally to expose heart. Insufflation of air-ether with remissions; air catheter through tracheal cannula.

Change pad to medium. Heart beats regular, rapid after opening pericardium. Both pleural cavities open; lungs pink, good distension; spontaneous respiration present.

+10.50. 0.47 cc. of adrenalin into tracheal cannula (air catheter temporarily withdrawn), 0.25 cc. per kilo.

10.51. Left ventricle beats half as fast as right ventricle, right auricle, and left auricle. Left ventricular contractions weaker than those of right.

10.54. Both ventricles beat synchronously, following auricles; left ventricle stronger beat than before. No foam in trachea.

11.01. Chambers beat synchronously; good strength.

+11.03½. 0.47 cc. of adrenalin, into trachea.

11.04. Left ventricle half as fast as right ventricle; left auricle very large now; both auricles and right ventricle beat at same rate; left ventricle at half that rate and contractions weak.

11.06. As before, but left ventricle beats more strongly.

11.06½. Left ventricle shows strong and weak beat alternately.

11.08. Left ventricle now beats at same rate as right ventricle; left auricle much smaller now.

Animal killed by clamping the heart. On excising the lungs, the right side is large, full, rounded, with no emphysematous blebs on surfaces; the left side collapses fully. Right lung shows a good amount of foam in the alveoli and large bronchi; the left lung exhibits only slight edema. The surfaces show a moderate number of pin-point hemorrhages. Heart beats vigorously after excision; there are numerous hemorrhages over the left and right ventricles. Left auricle shows extensive hemorrhage on inner surface. Right ventricular endocardium shows a fair number of hemorrhages, only a few in left ventricle. Heart swiftly passes into rigor.

The protocol given above does not, however, show all the gross changes which may be observed in a series of experiments by inspection of the heart. The change of the left ventricular rate to half of that of the right ventricle is usually preceded by a stage in which the left ventricle shows an alternation of a weak and a strong beat. Then the weak beat becomes no longer detectable to the eye and the left ventricle apparently beats at half the right ventricular rate. The left auricle usually beats at the same rate as the right half of the heart, but in one instance its rate was that of the left ventricle· the right half of the heart thus contracted twice to one contraction of the left half. This alteration in the functional activity of the left ventricle was noted in six of the seven experiments. The duration varied from a few seconds to 3 minutes.

In a few instances the entire heart stopped for the length of a beat or two, which accounts for the abrupt blood pressure drops mentioned previously. The dilatation of the left auricle was often tremendous, and made this structure look like a bright red blister. This dilatation is not dependent upon section of the vagi, for a sim-

ilar large dilatation of the left auricle with marked swelling of the aorta and pulmonary artery was also noted in the single experiment of this series in which the vagi were intact.

The pulmonary artery may dilate tremendously after the intratracheal injection of adrenalin; the dilatation develops shortly after the injection and may last 7 minutes. This dilatation is just as marked during diastole of the right ventricle as during its systole. Dilatation of the pulmonary artery was noted in three experiments, not looked for in three, and not seen, though sought for, in one experiment.

The aorta dilated markedly shortly after the adrenalin injection in five experiments and its condition was not observed in two.

In two experiments the adrenalin seemed to exert a locally inhibiting effect upon the muscle of the ventricle. In one instance the upper half only of the right ventricle contracted during systole, the lower half dilating. In this experiment the state of the aorta and pulmonary arteries unfortunately was not observed, but the left auricle dilated enormously and the left ventricle showed the changes in beat already described.

In the second instance the musculature of the left ventricle was affected locally, and with each systole a prominent though small, bright red bulging appeared near the apex. In this animal the aorta and left auricle were strongly dilated; the left ventricle showed the changes in character and rate of beat described previously; the pulmonary artery was not observed. The pulmonary edema obtained in this animal was only slight.

In this series of experiments with double vagotomy and exposure of the heart under artificial respiration none of the animals died acutely, but all were killed after at least 20 minutes had elapsed, by clamping the heart or by medullary puncture. Moreover, the pulmonary edema observed in the excised lungs was only slight in four, and fair to moderate in the other three experiments.

In these experiments the right auricle and right ventricle showed no marked dilatation at any time, nor was any dilatation of the left ventricle observed which could account for the swelling of the left auricle by regurgitation through the mitral valves. On the contrary, the left ventricle was in a state of greater tone with small systolic and diastolic excursions, so that the distension of the left auricle was caused by the inability of this chamber to empty all the blood it received from the lungs into the tonically contracted left ventricle.

Although these experiments yielded interesting information concerning the activity of the heart after the repeated administration of adrenalin in vagotomized animals, the main result is the suggestive absence of any considerable pulmonary edema. This is the more interesting because all the conditions apparently favored the production of pulmonary edema: pressure in the pulmonary veins was surely raised because the dilatation of the left auricle shows that it received more blood than it could handle, so that back pressure resulted; the pressure in the pulmonary artery was also raised as shown by its dilatation; and still further proof of increased pressure in the pulmonary circulation is furnished by the development of hemorrhages during inspection of the lung.

As these experiments were necessarily carried out under artificial respiration, a small series of tests was made to study the effect of artificial respiration upon the production of pulmonary edema when the chest was intact. Four rabbits were utilized; their vagi were divided and intratracheal insufflation started with air-ether in various proportions; in one animal air only was given after the operation had been completed. The adrenalin dose was 0.25 cc. per kilo intratracheally, administered once. In this series one animal died 17 minutes after the injection without any convulsions or foam in the trachea. Death was due to too much ether; autopsy showed pregnancy and only a slight degree of pulmonary edema. The other three animals were killed by medullary puncture 25 minutes after the adrenalin was injected, and the lungs were examined at once. None of them showed a marked degree of pulmonary edema, but some was present in all, especially in the lower lobes.

These results thus indicate that artificial respiration has a restraining effect upon the formation of pulmonary edema, and corroborate a similar observation made by Emerson.[6] How this protection is possibly exerted will be discussed later.

Tracheal Stenosis.

In another set of experiments the effect of an inspiratory decrease of pressure in the lung alveoli upon the production of pulmonary

[6] Emerson, H., *Arch. Int. Med.*, 1909, iii, 368.

edema in animals with vagi intact was tested. This was accomplished by compressing the trachea with a clamp. The air now entered so slowly during inspiration that a rarefaction of the intrapulmonic air occurred, as was evidenced by the sinking in of the costal margins and of the lower part of the sternum. The lung alveoli thus must exert some suction during inspiration upon the capillaries in their walls, and this should aid in the production of an adrenalin pulmonary edema in normal rabbits (vagi intact).

Fifteen experiments were made, of which ten were controls. The adrenalin animals received 0.3 cc. of adrenalin per kilo intratracheally; after about 2 minutes the trachea was stenosed. About 15 minutes after the injection the animals were killed and the lungs examined at once.

In 5 rabbits where adrenalin had been injected intratracheally and the trachea stenosed, 4 exhibited a good or well marked pulmonary edema; in 1 the edema was slight. The edema, however, was never as great as that seen in vagotomized animals.

In 5 controls, adrenalin was injected in the same dosage, but the trachea was merely exposed, but not constricted. 4 of these rabbits showed slight or no edema, but the fifth exhibited a well marked pulmonary edema. This latter animal, however, was abnormal, for both peritoneal and pleural cavities contained a large amount of clear straw-colored fluid.

In the second set of controls no adrenalin was injected but the trachea was constricted and the animal killed by asphyxia 15 minutes after beginning the stenosis. Of these five animals, all showed either slight or no edema of the lungs. It is seen that stenosis of the trachea and final asphyxia do not suffice in the normal rabbit to bring about pulmonary edema.

This series of experiments thus shows clearly that the cupping action of the lung alveoli on the pulmonary capillaries during inspiration plays a part in the production of adrenalin pulmonary edema.

Distribution of the Pulmonary Edema.

The edema obtained after vagus section was not uniform in its distribution throughout the lung. Invariably the lower lobes were much juicier and more hemorrhagic than the middle and upper lobes. On section the heavy, lower

lobes resembled sponges soaked to saturation with a pink fluid; squeezing usually yielded little foam but much fluid. The middle and upper lobes showed chiefly white foam with little fluid; occasionally, however, the middle and upper lobes also contained much fluid.

Hemorrhages also were much more extensive over the surfaces of the lower lobes than over the upper lobes (Fig. 2). The outer third of the upper lobes was usually free from hemorrhages.

As a rule, the right and left halves of the lungs were equal in size, but in a number of instances the right side was appreciably larger than the left, and in some of these cases the pulmonary edema was also greater in the larger half.

Atropine Series.

This series of experiments was undertaken in order to test the effect of adrenalin after paralysis of the motor vagus endings. The animals were tracheotomized, the vagi divided, and atropine sulfate was injected in 1 per cent solution about 5 minutes before the adrenalin. In some cases the blood pressure was recorded. The thorax was intact, and no artificial respiration was given. The dose of atropine varied between 1 and 2 mg. per kilo; the amount of adrenalin was always 0.25 cc. per kilo, injected intratracheally. The atropine was injected intramuscularly in the first three animals but in the remaining seven it was administered intratracheally.

Of 3 animals which received atropine intramuscularly, 2 died acutely in 10 to 14 minutes after a single injection of adrenalin showing typical symptoms of pulmonary edema. The other rabbit, however, gave no clinical signs of pulmonary edema within 1 hour, during which he received not one but three doses of adrenalin, each 0.25 cc. per kilo, at 15 to 20 minute intervals. Death occurred after $2\frac{1}{2}$ hours and the lungs exhibited a marked pulmonary edema. This last animal received a larger dose of atropine than the two preceding ones; the first two received 1 and 1.25 mg. respectively per kilo, while the third rabbit was injected with 1.5 mg. per kilo.

In the other seven experiments the atropine was injected intratracheally, the first dose tested being 1.25 mg. per kilo. This amount, in subsequent tests, was increased until 2 mg. per kilo were given. In this series the results were encouraging. 1 animal showed foam and fluid in the trachea, but was in fair condition 35 minutes after the adrenalin injection. The other 6 showed no clinical sign of edema,

the respiration was easy, no râles were palpable or audible, and no foam or fluid was ever seen during the 30 to 40 minutes of observation. All the animals were then killed by medullary puncture and the lungs examined: 5 animals showed slight or no edema, 1 showed moderate edema, and 1 exhibited a marked degree of pulmonary edema. This animal was the one in which foam and fluid issued from the trachea 15 minutes after the adrenalin injection.

Five control experiments without atropine were made with animals from the same lot. Of the 5, 2 died within 10 minutes and the remaining 3 succumbed within 20 minutes after the adrenalin injection. The lungs of all showed a marked degree of pulmonary edema. The differences between lungs of these two series is well brought out by Figs. 2 and 3.

The following table summarizes the results of the atropine series and its controls.

Atropine Series. Vagi Divided. All Injections Intratracheal.

No. of animals used.	Atropine per kilo.	Adrenalin per kilo.	Death in 10 min.	Death in 20 min.	Autopsy.
	mg.	*cc.*			
7	1.25–2.00	0.25	0	0	Slight or no pulmonary edema in 5 animals; moderate pulmonary edema in 1; marked pulmonary edema in 1.
5	None given.	0.25	2	3	Marked pulmonary edema in all.

The protective action of atropine against adrenalin pulmonary edema in normal animals has been noted by Biedl.[7]

DISCUSSION.

In the previous pages we have shown that adrenalin pulmonary edema occurs more frequently, in a more severe form, and after a smaller dose in rabbits if the vagi are sectioned previous to the intratracheal administration of the drug, than when the adrenalin is given in the same way to rabbits with vagi intact. It has been pointed

[7] Biedl, A., Innere Sekretion, Berlin, 2nd edition, 1913, i, 522.

out that artificial respiration strongly reduces the degree of pulmonary edema after adrenalin when the vagi have been divided, and this result is obtained both with opened and intact chests. Data have been furnished which indicate that after adrenalin administered intratracheally the heart ventricles temporarily deliver unequal amounts of blood with each systole, the right ventricle delivering more blood than the left, shown by the tremendous dilatation of the left auricle and by the marked swelling of the pulmonary artery, which remained distended during diastole as well as systole; it has also been shown that adrenalin produces a temporary incoordination of the heart so that the left ventricle beats at one-half the rate of the right auricle and ventricle. Finally we have demonstrated that atropine markedly reduces the occurrence of pulmonary edema after vagus section and adrenalin.

What bearing do these facts have on the production of pulmonary edema? Leaving aside for the moment the striking adjuvant action of vagus section in the formation of pulmonary edema, it will be noted in the first place that the cardiac changes observed by us after the administration of adrenalin are in full accord with the fundamental postulate of Welch's theory[8] for the production of a general acute pulmonary edema. It will be remembered that this is a disproportion between the ventricular outputs of such a character that the left ventricle expels less blood per systole than the right, thus producing an acute venous hyperemia of the lungs. In our experiments on rabbits whose hearts were exposed for inspection (artificial respiration) we saw this demand fulfilled: shortly after the intratracheal injection of adrenalin the pulmonary artery dilated widely and remained dilated during each diastole of the heart; at the same time the left auricle swelled tremendously and sooner or later the left ventricle showed a rate which was only half that of the right side of the heart, and its amplitude of contraction was small. These facts indicate unquestionably that the right heart is acting vigorously, that the arterial and venous pressures in the lung are

[8] Welch, W. H., *Virchows Arch. path. Anat.*, 1878, lxxii, 375. See also his later presentation in S. J. Meltzer's Harrington Lectures on Edema, *Am. Med.*, 1904, viii, 37 of reprint.

raised, and finally that the left heart is surely expelling less blood per systole than the right.[9]

The physical conditions for the production of pulmonary edema demanded by Welch are therefore present, and yet the pulmonary edema which we obtained in this series with vagi divided, where the heart was exposed under artificial respiration, was slight and even negligible when compared with the tremendous edema which resulted when the same dose of adrenalin was injected into animals (vagi divided) whose thorax was intact and which were breathing in the normal way. Artificial respiration thus seemed to be an inhibitory factor, a supposition which was strengthened by the results of another series of experiments with chest intact, where the vagi were divided and intratracheal air insufflation with rhythmical remissions of the pressure was given throughout. Here again no marked degree of pulmonary edema was observed after the injection of adrenalin, either clinically, or after killing the animal and examining the lungs. Moreover, there are statements in the literature which furnish direct and indirect evidence regarding the effect of artificial respiration on pulmonary edema.

Emerson[6] in a brief note reports that gentle artificial respiration, with or without suction, produces an amelioration of the pulmonary edema in cats, caused by the intravenous injection of massive doses of adrenalin. That artificial respiration may even prevent adrenalin pulmonary edema in rabbits follows directly from a statement of Miller and Matthews[10] that they were never able to obtain pulmonary edema in a considerable number of rabbits by injecting intravenously 0.5 to 2 cc. of 1: 1,000 adrenalin. Now, 1 to 2 cc. of adrenalin, intravenously administered, are sure to cause pulmonary edema in a percentage of normal animals provided that the number tested is not too small, and Miller

[9] While our experiments show only that a rise of pressure undoubtedly exists in the pulmonary circulation, it might be assumed that it is due solely to back pressure from the left auricle. There is, however, good evidence to show that adrenalin contracts the pulmonary blood vessels, and without entering into the question of pulmonary vasomotors, the following observers, who have noted a rise of pressure in the pulmonary artery or a constriction of the pulmonary vessels after adrenalin, may be mentioned: Weber, E., *Arch. Physiol.*, 1910, Suppl., 410; 1912, 383; 1914, 535. Führer, H., and Starling, E. H., *J. Physiol.*, 1913–14, xlvii, 301. Tribe, E. M., *ibid.*, 1914, xlviii, 154.

[10] Miller, J. L., and Matthews, S. A., *J. Physiol.*, 1909, iv, 370.

and Matthews state that they used a "considerable number." But artificial respiration was necessary in their experiments to record the pressure in the pulmonary artery, and in view of the statements already made regarding the action of this procedure on the production of pulmonary edema, their failure to obtain edema is readily explained. Hallion and Nepper[11] also give as an impression that acute pulmonary edema in rabbits after the intravenous injection of large doses of adrenalin occurred apparently less readily when the thorax was open than when the chest was intact. They are inclined to attribute this action to mechanical conditions of the experiment, circulatory changes in the lung, and they also mention as a factor the positive intraalveolar pressure produced by artificial respiration.

The question now arises how artificial respiration reduces or prevents the pulmonary edema called forth by adrenalin in rabbits.

Artificial respiration does not prevent cardiac changes resulting in an acute congestion of the lungs, for we have observed, as described previously, such cardiac changes in rabbits when the chest was open. Emerson's tentative explanation[12] that the distension of the lung by artificial respiration drives a considerable amount of blood into the left auricle thus relieving the pulmonary congestion, is unsatisfactory even for the conditions theoretically deduced or experimentally observed by him, for he states that the adrenalin causes acute dilatation of the left ventricle with consequent mitral regurgitation, acute congestion of the lungs, and a dilatation and failure of the right heart; edema results due to the back pressure from the left ventricle. On the basis of this conception it is impossible for us to see how the massage of blood by artificial respiration from the lung into an acutely dilated left ventricle with incompetency of the mitral valves, could reduce the back pressure which, according to Emerson, causes the edema. In this connection we may say that we did not observe any acute dilatation of the left ventricle or regurgitation into the left auricle, nor have we seen any marked dilatation of the right ventricle during the cycle of cardiac changes induced by adrenalin. The left ventricle in the rabbit after adrenalin always seemed in a state of greater tone than normal. A few times the entire heart apparently stopped for a few beats in diastole but without any resulting marked dilatation. We are inclined therefore to believe that

[11] Hallion and Nepper, *J. physiol. et path. gén.*, 1911, xiii, 893.

[12] Emerson,[1] p. 370.

artificial respiration with positive pressure does not exert its inhibitory action on pulmonary edema through an action on the heart itself.

There is a factor, however, in the production of pulmonary edema, which is abolished by ordinary artificial respiration, that has received practically no consideration so far as we know. That factor is the aspirating action of the lung alveoli under certain conditions during inspiration. When the diaphragm descends, the negative pressure in the thorax increases, and air enters freely into the alveoli through the trachea and bronchi. The air pressure in the alveoli and consequently upon the capillaries in the alveolar walls remains at atmospheric level. If, however, there is a partial or even complete obstruction in the bronchioles, for example, by contraction of the bronchial muscles, hindering or preventing the entrance of air, then during each inspiration every alveolus connected with such a constricted bronchiole must act like a miniature dry cup, for the pressure in the alveoli and on the capillaries in their walls must decrease as these elastic chambers expand due to the disproportion between intra-alveolar and intrathoracic pressures. If in addition there is a congestion in the pulmonary circulation the passage of a transudate into the alveoli is surely facilitated if not even initiated by this pressure decrease in the alveoli. Experimental support for this view is given by the series of experiments where adrenalin was injected in rabbits whose tracheæ were stenosed.

This conception that the lung alveoli may act like dry cups, has been mentioned as early as 1845 by Mendelsohn.[13] It must be added, however, that this action of the pulmonary air cells occupies but a subsidiary part in Mendelsohn's elaborate development of the thesis that lung hyperemia in general depends upon diminished expansion and contraction of the lungs during respiration. For, according to Mendelsohn, distension of the lungs stretches the pulmonary artery and its capillaries, increasing their volume capacity and thus aspirating blood; any diminution of the expansion and contraction diminishes this aspiration and reduces the pulmonary circulation which now depends solely upon the propulsive power of the right ventricle, so that stasis occurs.[14]

[13] Mendelsohn, *Arch. physiol. Heilk.*, 1845, iv, 277.

[14] See also Mendelsohn's final publication, Der Mechanismus der Respiration und Cirkulation, oder das explicierte Wesen der Lungenhyperämien, Berlin, 1845.

Is there any evidence that such a constriction of the bronchioles occurs after the injection of adrenalin? Since the publication of Kaplan's[15] observation that adrenalin relieves bronchial asthma, most of the investigators describe a relaxation of the bronchial muscles as the result of an adrenalin injection,[16] yet bronchial constriction by the same substance has also been noted. Golla and Symes[17] with a new method of artificial respiration obtained constriction of the bronchioles in decerebrate cats and rabbits after adrenalin unless the bronchioles were initially constricted by other drugs, under which condition a relaxation was obtained. One of us, in some unpublished work, has also observed oncometrically a definite decrease in the amplitude of the lungs in rabbits after the intravenous injection of adrenalin, and it has already been stated that we have often seen a distension of the excised rabbit lung after adrenalin which was not accounted for by the degree of pulmonary edema present.

There is thus sufficient experimental proof that adrenalin may cause a constriction of the bronchial muscles and it is evident that as soon as this occurs the cupping action of the alveoli during inspiration can take place, thus aiding the passage of fluid from the gorged capillaries into the alveoli.

On the basis of this action of adrenalin, the protective action of artificial respiration may be explained; the positive pressure which artificial respiration produces in the lung reduces or overcomes the bronchial constriction due to the adrenalin and thus prevents the intraalveolar pressure from becoming negative during inspiration. Consequently the cupping action on the pulmonary capillaries does not occur. Thus one link in the chain of conditions which are more or less necessary for the production of pulmonary edema is broken, and edema is then produced with greater difficulty and to a less extent.

The protective action of atropine against pulmonary edema from adrenalin after vagus section may also be explained by paralysis of the bronchomotor endings of the vagus, and the consequent inability

[15] Kaplan, D. M., *Med. News*, 1905, lxxxvi, 871.

[16] Januschke, H., and Pollak, L., *Arch. exp. Path. u. Pharm.*, 1911, lxvi, 206–214. Trendelenburg, P., *ibid.*, 1912, lxix, 104. Jackson, D. E., *J. Pharm. and Exp. Therap.*, 1912–13, iv, 74, 291; 1913–14, v, 509. Baehr, G., and Pick, E. P., *Arch. exp. Path. u. Pharm.*, 1913, lxxiv, 62, 71. Dixon, W. E., and Ransom, F., *J. Physiol.*, 1912–13, xlv, 413.

[17] Golla, F. L., and Symes, W. L., *J. Pharm. and Exp. Therap.*, 1913–14, v, 88.

of adrenalin to bring about constriction of the bronchioles, thus preventing the aspirating action of the alveoli. This is in accord with the prevailing view concerning the point of attack of both atropine and adrenalin. It must be mentioned, however, that Golla and Symes[18] report that 10 mg. of atropine in a rabbit failed to relieve the bronchoconstriction caused by adrenalin. It is of course obvious that atropine may also affect the heart or the lung vessels and endothelia in such a way as to prevent or reduce pulmonary edema. But as we have no direct experimental evidence of our own we shall not enter into a discussion of these possibilities.

The aspirating action of the lung alveoli during coexisting pulmonary congestion also explains why the edema is always greater in the lower lobes than in the middle and upper lobes. This is due to the fact that the negative pressure is greater in the lower lobes than in the others. Experimental evidence for this is furnished by the work of Meltzer[19] and Meltzer and Auer,[20] which indicates that the negative pressure in the chest is not the same at all levels but is greatest in the lower portion of the thoracic cavity. If a bronchiolar constriction occurs during a marked hyperemia of the lungs it follows that the cupping action of the alveoli during inspiration must be most effective where the negative pressure variation is greatest, provided that the hyperemia and endothelial permeability are the same throughout the lung.

We come now to a consideration of the remarkable accelerating effect which vagus section exerts upon the production of adrenalin pulmonary edema, and it may be stated at once that we cannot offer a full analysis because the experimental test of the various possibilities is incomplete. It may be said, however, that this difference in action is apparently a quantitative one, for pulmonary edema can usually be obtained in the normal rabbit provided that sufficient adrenalin is administered without causing cardiac death. The optimum state for the occurrence of a strong pulmonary edema develops more slowly in a rabbit with vagi intact than in one with vagi sectioned. This suggests that the structure primarily responsible for

[18] Golla and Symes,[17] p. 93.

[19] Meltzer, S. J., *J. Physiol.*, 1892, xiii, 218.

[20] Meltzer, S. J., and Auer, J., *J. Exp. Med.*, 1910, xii, 34.

the fulminant onset of edema is the heart. By vagus section the heart is deprived of important regulating influences, and incoordination of the two sides of the heart perhaps then results more readily under stress; that such an incoordination does take place promptly when the vagi are divided and adrenalin is administered, we have already shown. Our experiments on the action of adrenalin on the fully innervated exposed heart of the rabbit are too few to permit any conclusion. The electrocardiographic studies on the action of adrenalin in the dog made by Kahn[21] and by Rothberger and Winterberg[22] which showed no incoordination of the heart after the vagi were sectioned (Kahn), but elicited changes in the form of waves very similar to those obtained by faradic stimulation of the accelerators (Rothberger and Winterberg), tend to support our view because the dog after vagus section tolerates large doses of adrenalin without developing pulmonary edema.

Another action which vagus section may facilitate is the production of bronchial constriction by adrenalin. After vagus section the bronchial muscles are relaxed and thus in the optimum state for contraction as demanded by Golla and Symes. The adjuvant action of bronchial constriction in the production of pulmonary edema has already been discussed.

Vagus section not only facilitates the formation of pulmonary edema after adrenalin, but it is an indispensable preliminary for obtaining pulmonary edema in experimental hydremic plethora.

Kraus[23] reports that the infusion of large quantities of salt solution in cats and rabbits only caused pulmonary edema when the vagi were sectioned. He also observed that adrenalin aided in the production of this edema; that open or closed thorax exerted no influence; and that atropine did not prevent this form of edema. Kraus assumes tentatively that section of centripetal vagus fibers, which govern reflexly the lung vasomotors, causes a disturbance in the regulation of the blood supply to the lung leading indirectly to edema.

Although Kraus' experiments and our own were carried out under widely different conditions so that a comparison cannot be made,

[21] Kahn, R. H., *Arch. ges. Physiol.*, 1909, cxxix, 379.
[22] Rothberger, J., and Winterberg, H., *Arch. ges. Physiol.*, 1910, cxxxv, 531.
[23] Kraus, F., *Z. exp. Path. u. Therap.*, 1913, xiv, 402.

it is nevertheless highly suggestive to note than in both sets of experiments vagus section exerted a profound influence. The nature of this influence cannot be stated with certainty at the present time.

In the preceding pages we have made no attempt to give a complete presentation of the problem of pulmonary edema but have limited ourselves largely to those facts which follow directly from our experiments. Necessarily, therefore, numbers of factors have been touched only lightly or not at all in the discussion.

SUMMARY.

The intratracheal injection of one moderate dose of adrenalin in rabbits whose vagi are divided produces a marked pulmonary edema in a large percentage of cases. The same dose in normal animals causes only slight effects.

Artificial respiration greatly reduces the production of pulmonary edema in vagotomized rabbits after adrenalin.

As adrenalin can exert a bronchoconstrictor effect, evidence is submitted to show that the aspirating action of the lung alveoli under this condition apparently plays an important part in the production of adrenalin pulmonary edema. On the basis of this mechanism the protective action of artificial respiration is explained.

Stenosis of the trachea facilitates the production of adrenalin pulmonary edema in rabbits whose vagi are intact.

The intratracheal injection of adrenalin in vagotomized rabbits produces a temporary incoordination between the heart ventricles, visible on inspection, so that the left ventricle beats apparently half as fast as the right, causing hyperemia of the lungs and hemorrhages.

Atropine injected intratracheally in vagotomized rabbits exerts a protective action against adrenalin pulmonary edema.

EXPLANATION OF PLATES.

PLATE 14.

FIG. 1. Blood pressure record. Rabbit 3; black female. Weight 1,980 gm. Both vagi were divided. The time is marked in 4 second intervals. The time line is also the zero pressure level. 0.5 cc. of adrenalin (0.25 cc. per kilo) was injected into the tracheal glass cannula. Note the three abrupt drops in the blood pres-

sure with prompt recovery. The low level (25 to 50 mm. of mercury) was maintained for 20 to 60 seconds, and convulsions appeared during this time. The typical foam of pulmonary edema appeared in the trachea after the second fall, and pink fluid during the terminal descent of the blood pressure. The terminal fall was slowed by a partial clot. These abrupt pressure drops are possibly caused by a complete cardiac stoppage such as was observed when the heart was exposed for inspection.

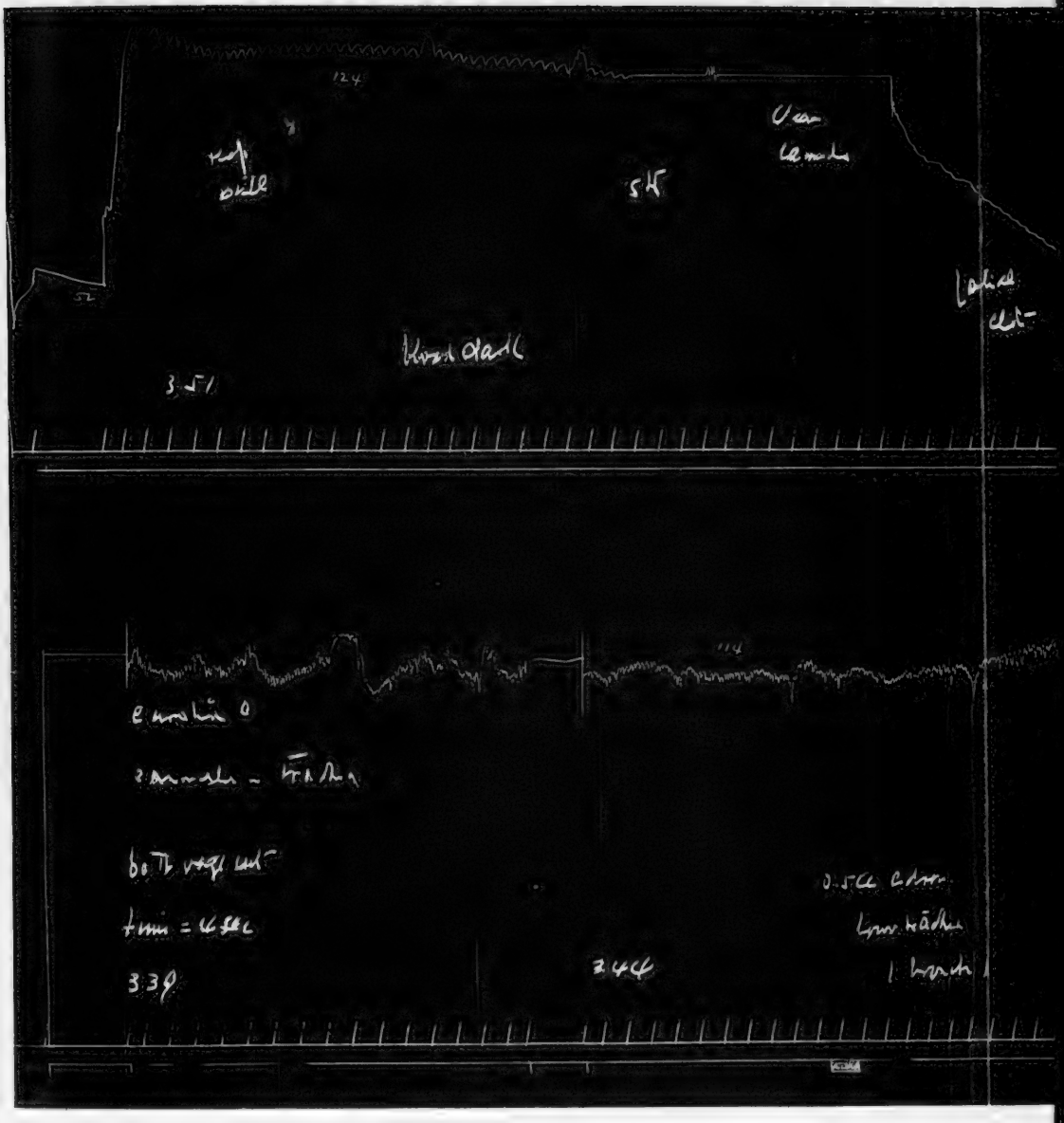
PLATE 15.

Figs. 2 and 3 show the protective effect of atropine. In both rabbits the vagi were divided and both received 0.25 cc. of adrenalin per kilo intratracheally. Rabbit 5, however, was injected with 1.75 mg. of atropine sulfate per kilo intratracheally before the adrenalin was administered.

FIG. 2. Rabbit 4, adrenalin alone. Typical pulmonary edema developed, with death in 18 minutes. The lungs were large, heavy, erect, hemorrhagic, with foam and fluid in the tracheal cannula.

PLATE 16.

FIG. 3. Rabbit 5, which received atropine before the adrenalin, showed no symptoms of pulmonary edema, and was killed after 45 minutes. The lungs collapsed practically normally, but the surfaces were peppered with small hemorrhages. The trachea showed no foam or fluid.



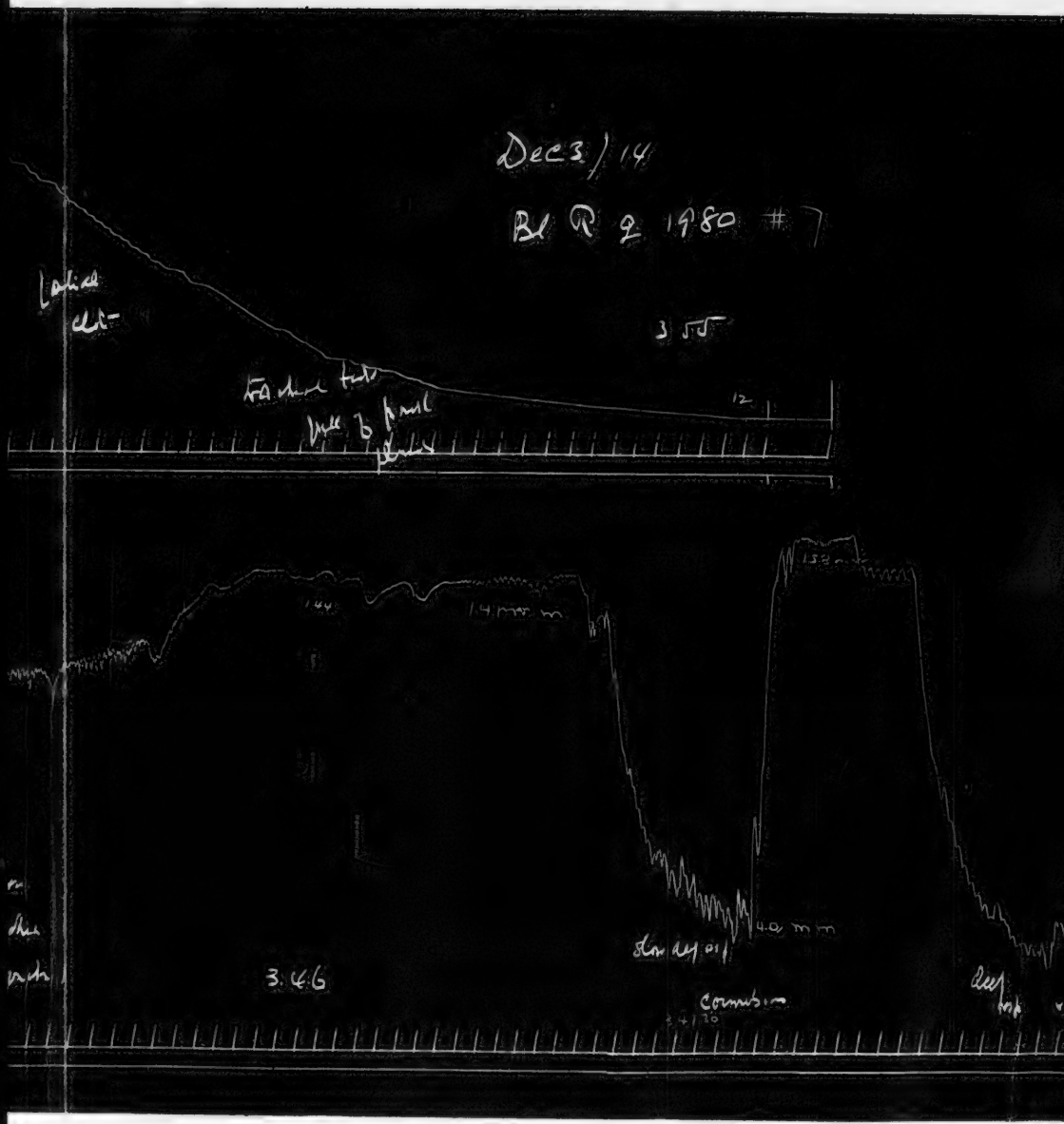
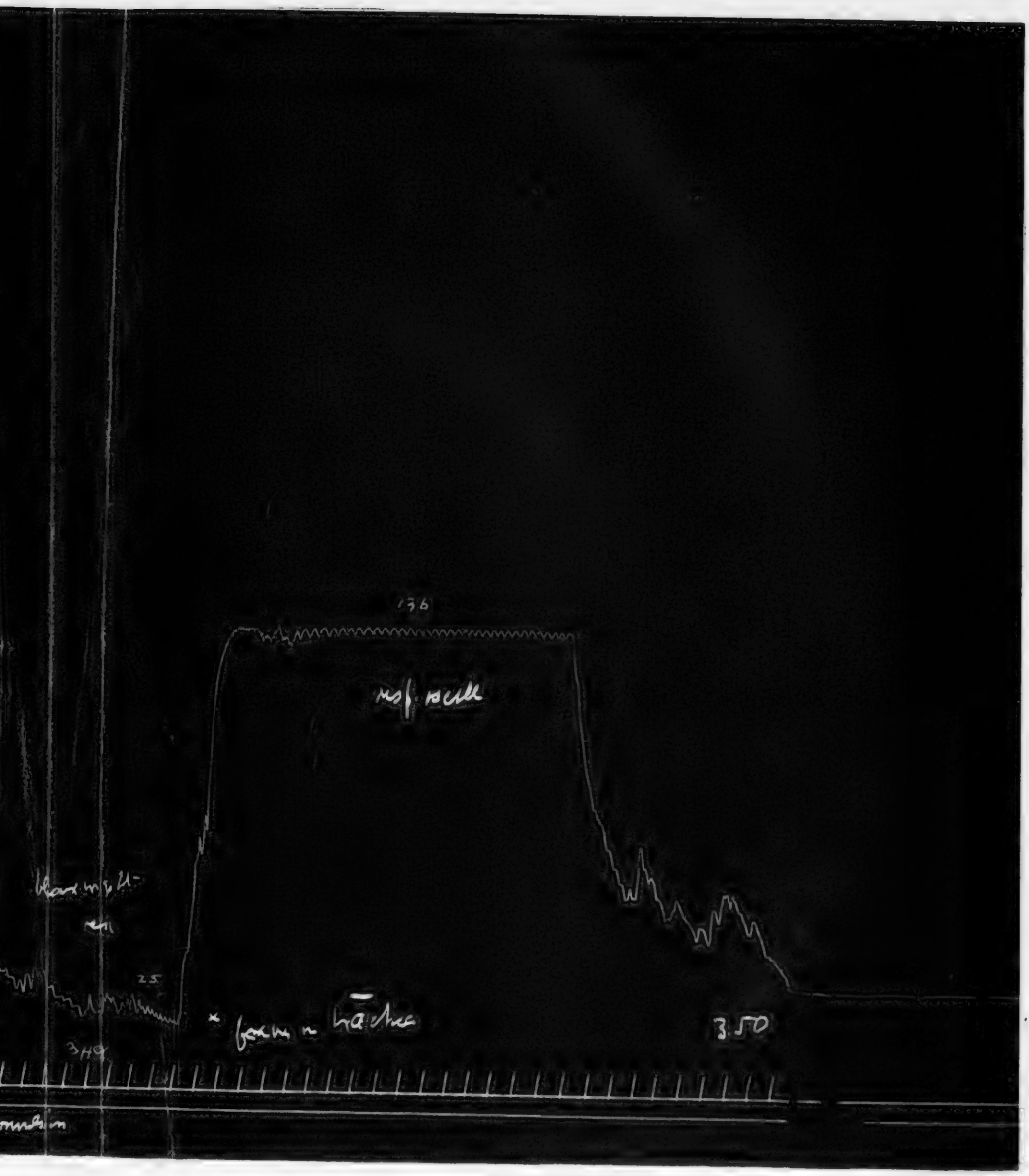


FIG. 1.

PLATE 14.



(Auer and Gates: Adrenalin pulmonary edema.)

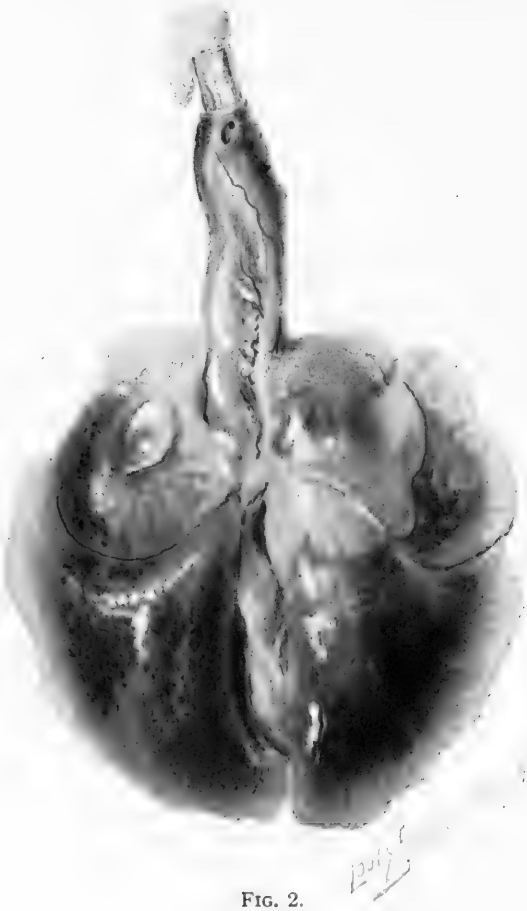


FIG. 2.

(Auer and Gates: Adrenalin pulmonary edema.)

FIG. 3.

(Auer and Gates: Adrenalin pulmonary edema.)

THE EFFECTS OF EXPERIMENTAL PLETHORA ON BLOOD PRODUCTION.

By OSWALD H. ROBERTSON, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, March 22, 1917.)

Our present knowledge concerning normal blood production indicates that the bone marrow functions at a constant rate supplying new red corpuscles to replace those lost daily in the process of normal blood destruction. Since an extra loss of blood causes greater activity of the bone marrow,[1] it has seemed possible that were the constantly occurring normal loss compensated for artificially there would be no necessity for the production of new cells, and consequently a lessened activity of the bone marrow might result. In an attempt to bring about this condition, rabbits have been made plethoric by repeated small transfusions of blood and the effect of the procedure on the bone marrow has been observed.

Some work has already been done in this direction. Hess,[2] with the purpose of determining the effect of plethora on the heart, gave rabbits repeated large transfusions. At the end of 2 months of plethora the rabbits were killed. In examining the bone marrow, he claimed to have found evidence of a markedly diminished activity. The erythroblasts and myelocytes were decreased in number, and there was a fibrous hyperplasia present. Itami[3] gives a more detailed account of these findings, agreeing with Hess in his conclusions. Boycott and Douglas[4] carried on a similar series of experiments but failed entirely to confirm Hess's and Itami's results, which seem to have been obtained in only a few animals.

[1] The term "bone marrow activity," as used in this paper, refers only to the erythropoietic function of the marrow.

[2] Hess, R., *Deutsch. Arch. klin. Med.*, 1909, xcv, 482.

[3] Itami, S., *Folia hæmatol.*, 1908, vi, 425.

[4] Boycott, A. E., and Douglas, C. G., *J. Path. and Bacteriol.*, 1909, xiii, 414.

The criteria which these two sets of investigators used as evidence of depressed bone marrow activity, that is, marked histological changes in the marrow, may account to some extent for their contradictory results, since the red marrow of even normal rabbits varies much in its morphology as indicative of blood production and also in its distribution.

In the present work, use has been made of an indicator which may be thought to represent more nearly the actual functional variations in the marrow taken as a whole; namely, the number of reticulated red cells in the circulating blood. In addition, the number of these cells in the bone marrow itself has been determined. In normal animals, reticulated red corpuscles are present in the blood in fairly constant numbers. There is good evidence that increased activity of the bone marrow is accompanied by an increase in the percentage of these cells, and that the percentage roughly parallels the degree of hyperfunction.[5,6] *A priori*, one would suppose that if the activity of the bone marrow were lessened, a drop in the reticulated cell count would result.

Method of Producing Plethora.

The method of producing the plethora has already been described[7] but will be briefly given here. Rabbits were used. Each recipient was provided with three to six donors of the same hemolytic group. Transfusions of 10 cc. of blood were made daily. The donors were used in rotation, so that each one lost at most only 10 cc. every 3 days. In this way the production of any considerable anemia in the donor rabbits was avoided. In some of these rabbits, the blood loss was made up so promptly that the hemoglobin percentage did not vary throughout the period of the bleedings; in others it dropped slightly. The blood for transfusion was obtained from the donor by cardiac aspiration into a syringe containing 1 cc. of a 1 per cent solution of sodium citrate in normal salt solution. This small amount of citrate, when well mixed with 10 cc. of blood, was sufficient to prevent coagulation for the few minutes required to introduce the blood into the marginal ear vein of the recipient. The recipient rabbits for the most part weighed from 1,500 to 1,800 gm. All were young rabbits. Both sexes were employed.

[5] Lee, R. I., Minot, G. R., and Vincent, B., *J. Am. Med. Assn.*, 1916, lxvii, 719.
[6] Vogel, K. M., and McCurdy, U. F., *Arch. Int. Med.*, 1913, xii, 707.
[7] Robertson, O. H., and Rous, P., *J. Exp. Med.*, 1917, xxv, 665.

The Sahli hemoglobinometer was used for determining the degree of plethora. The inaccuracies of this method are recognized, but dependable readings were obtained by diluting for the color comparison after the test mixture had stood exactly 5 minutes. In some of the animals, the red cells were counted and the color index was estimated.

Method of Counting Reticulated Cells.

The following method for counting the reticulated cells was found to be preferable to those previously described. A saturated solution of brilliant cresyl blue was made up in normal salt solution. This was kept as a stock solution. When a count was to be made, a small quantity of it was diluted 80 times[8] with normal salt solution and mixed with blood in a pipette for counting white cells in the proportion of one part of blood to twenty parts of cresyl blue solution. The mixture was shaken in the pipette for 5 minutes. The cells were thus equally distributed as well as stained. They were counted at once in fresh preparations, which were sealed with vaseline to prevent disturbances due to drying. At least 1,000 red cells were counted at each test. When the number of reticulated cells was less than 1 in 1,000, 10,000 red cells were counted. In the latter case, only the first 1,000 were counted individually, the field being the unit of count for the remaining 9,000.

For several days before transfusions were begun, the number of reticulated cells was determined daily in those rabbits destined to be recipients.

In a large number of normal rabbits examined, the reticulated cells were found to vary for the most part between 10 and 20 per 1,000 erythrocytes. Rarely they were as many as 30 or fewer than 5 per 1,000. Only two animals showed less than 5 per 1,000, one having a count of 3 and the other of 4. The variation in the individual from day to day may be slight or, relatively speaking, considerable.

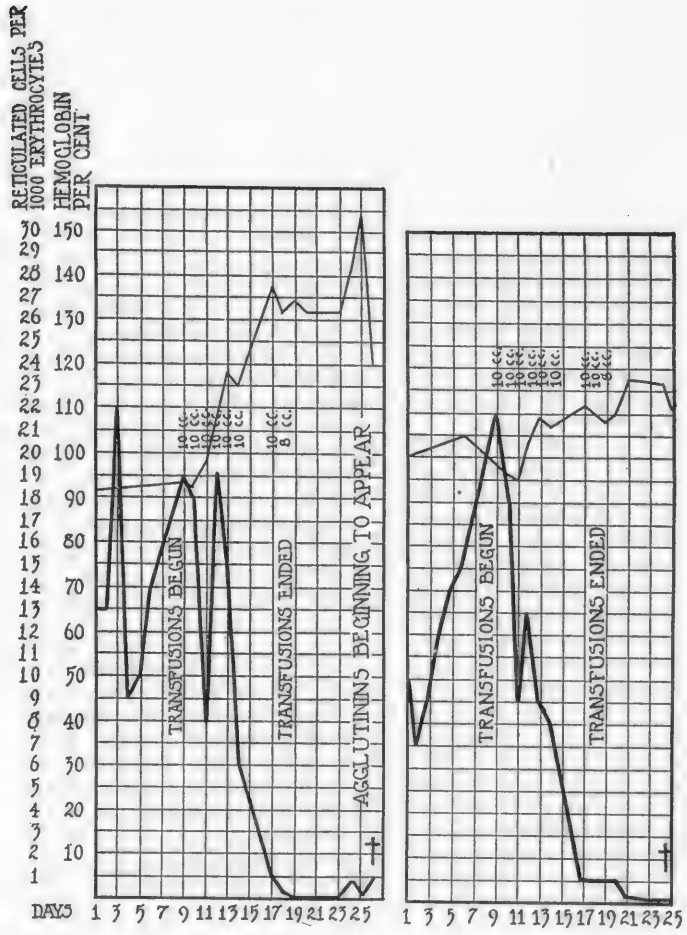
[8] Since doing this work, a second saturated solution of cresyl blue has been made up, using a different stock of the dye, which went into solution to a considerably greater extent than the first. The result was that a 1:80 dilution of this saturated solution was much too strong a staining fluid. It was found necessary to dilute to 180 for satisfactory staining. It is apparent, therefore, that each saturated solution of cresyl blue has to be tested beforehand for its optimum staining dilution. This is a very simple matter and needs to be done only once.

Production of Plethora.

Sixteen rabbits were rendered plethoric by the method described. The rate at which the hemoglobin per cent increased in different animals varied considerably. In some it rose rapidly. In others it increased slowly. The degree of plethora ultimately attained likewise varied much. An amount of blood which in one animal resulted in a rise of fifty points in the hemoglobin per cent, produced in another individual of the same size a rise of only thirty points. For this reason some of the animals were transfused more often than others. Even so it was often found impossible to force up the hemoglobin per cent in these refractory individuals to a height easily reached in other animals. The shortest period of transfusion was 9 days, during which the animal was given seven transfusions; the longest, 20 days with nineteen transfusions. As a rule, the hemoglobin per cent rose from a normal of 80 to 90 per cent to 140 to 150 per cent, at which point it remained fairly constant despite the continued introduction of blood.

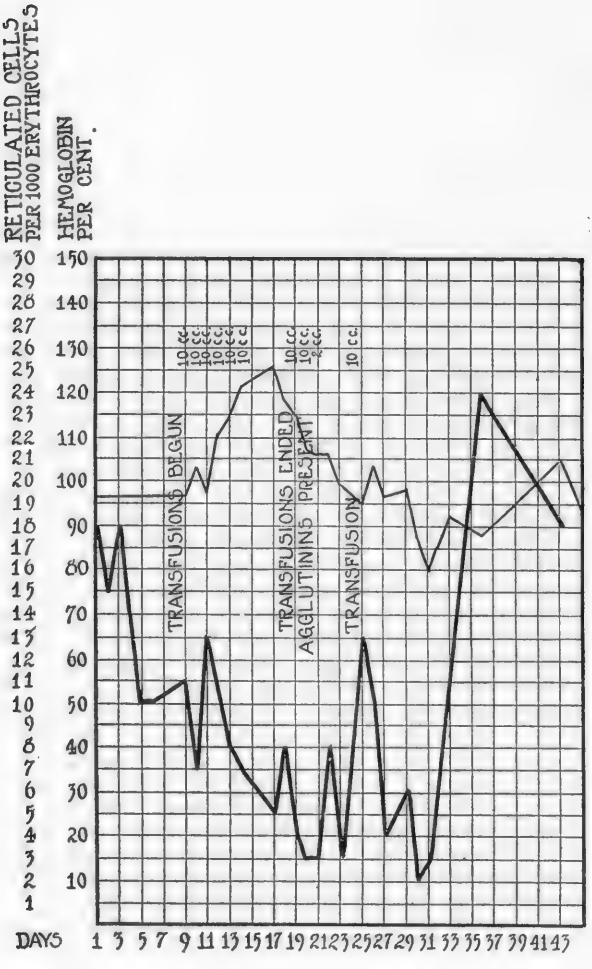
No notable change in the percentage of reticulated cells occurred until after several transfusions. Then, as plethora became well defined, the number of reticulated cells in the circulating blood began to diminish (Text-figs. 1 and 2). This decrease in number was sometimes rapid and soon became marked. Often the reticulated cells practically disappeared. Counts below 1 in 10,000 were frequent (Text-fig. 1), and sometimes a search through a whole slide would reveal none. The charts of Text-figs. 1 and 2 will illustrate the change that occurred in eight of the sixteen recipients. In five others, the drop in reticulated cells was less marked, though definite. They decreased to about 1 in 1,000. The degree of plethora was fully as great in these animals as in the afore mentioned ones, and no reason has been found for the differences observed.

Anomalous results were obtained in three rabbits. One showed only a slight drop in the reticulated red cells (Text-fig. 3). In the remaining two, these cells failed to decrease at all. One had a purulent pneumonia, with some anemia, despite the transfusions. The other developed an increased number of reticulated cells for which no cause was ever found.



TEXT-FIG. 1. TEXT-FIG. 2.

Reticulated cells.
Hemoglobin.


RETICULATED CELLS PER 1000 ERYTHROCYTES
HEMOGLOBIN PER CENT.
TRANSFUSIONS BEGUN
10 cc.
10 cc.
10 cc.
10 cc.
10 cc.
TRANSFUSIONS ENDED
AGGLUTININS PRESENT
10 cc.
10 cc.
2 cc.
TRANSFUSION
10 c.c.
DAYS

TEXT-FIG. 3.

It is evident from these results that a diminution in the number of reticulated cells in the circulation follows almost constantly the production of an artificial plethora. A possible influence of sodium citrate in causing this change has been ruled out by observations in five rabbits which received daily injections of sodium citrate alone, in some cases twice as much being given as in the rabbits made plethoric. The number of reticulated cells did not decrease in these animals.

Examination of the Bone Marrow.

The bone marrow from five of the eight rabbits in which the reticulated cells dropped markedly was examined for its content of these cells. The following procedure was employed.

Red marrow from the upper and lower ends of the femur was taken into a mixture of equal parts of Locke's solution containing ½ per cent of gelatin and an isotonic watery solution of sodium citrate. In this mixture clotting does not occur, and even the most delicate cells are well preserved. The gross appearance of the marrow was normal except for a congestion such as was present in all the other organs. A small portion of it was teased on a slide in a drop of the Locke's-citrate mixture and a count made at once from the preparation. Then, with a view to washing out the cells of the marrow, the needle of a syringe was thrust into it here and there, and gelatin-Locke's solution injected under considerable pressure. In this way a large part of the substance of the marrow was washed out. The washings were then centrifuged and counts made on the sedimented cells according to the technique used for the peripheral blood. To obtain a figure for purposes of comparison, an average was taken from the counts on the teased specimen and the washings.

The average number of reticulated cells in the bone marrow of the five plethoric animals was 14 per 1,000 red cells, the highest number 22 and the lowest 5 per 1,000. In striking contrast are the figures obtained in the examination of five normal rabbits. These showed an average of 320 per 1,000 red cells, the highest 540 and the lowest 160 per 1,000.

Anemia Following Plethora.

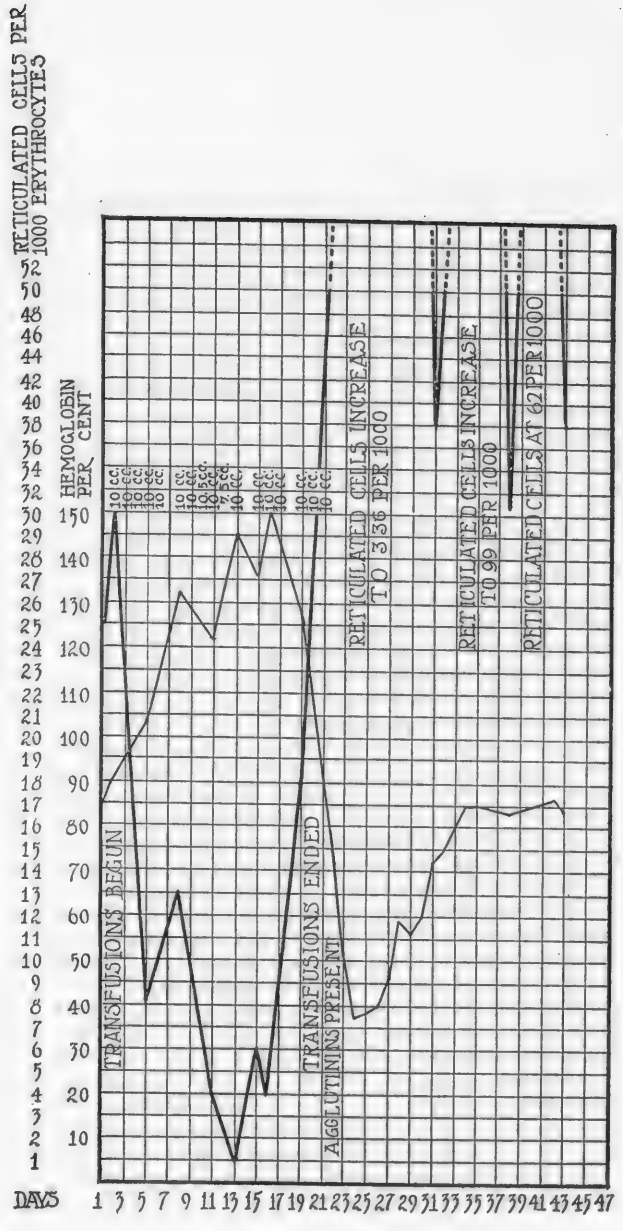
In certain of the plethoric rabbits there occurred, after many transfusions, a sudden marked drop in hemoglobin. In one instance (Text-fig. 4), despite daily transfusions of 10 cc. of blood, the hemo-

globin fell in 6 days from 150 per cent to 75 per cent. Transfusions were discontinued in such animals, and there rapidly ensued a severe anemia. This was observed in three rabbits. Charts of two of them are shown in Text-figs. 4 and 5. The cause of the rapid transition from plethora to anemia has not yet been determined, but it is significant that isoagglutinins for the donors' blood developed in all three rabbits at about the time when the hemoglobin first began to fall. The simplest explanation would seem to be that, following a number of transfusions, a lytic process develops against the strange cells, and they are rapidly destroyed. This results not merely in a return of the hemoglobin to normal but in an anemia, because of the peculiar condition of affairs indicated by the drop in reticulated red cells during the plethora. During it the bone marrow activity sinks far below normal, whereas blood destruction is maintained at the normal rate at least. Thus, little by little, as the animals' own cells are destroyed and not replaced, the bulk of the circulating blood comes to be strange blood liable to destruction when the lytic principle develops. The extent to which the blood cells proper to the recipient have been destroyed and replaced by alien cells is suddenly revealed through the destruction of this alien blood.

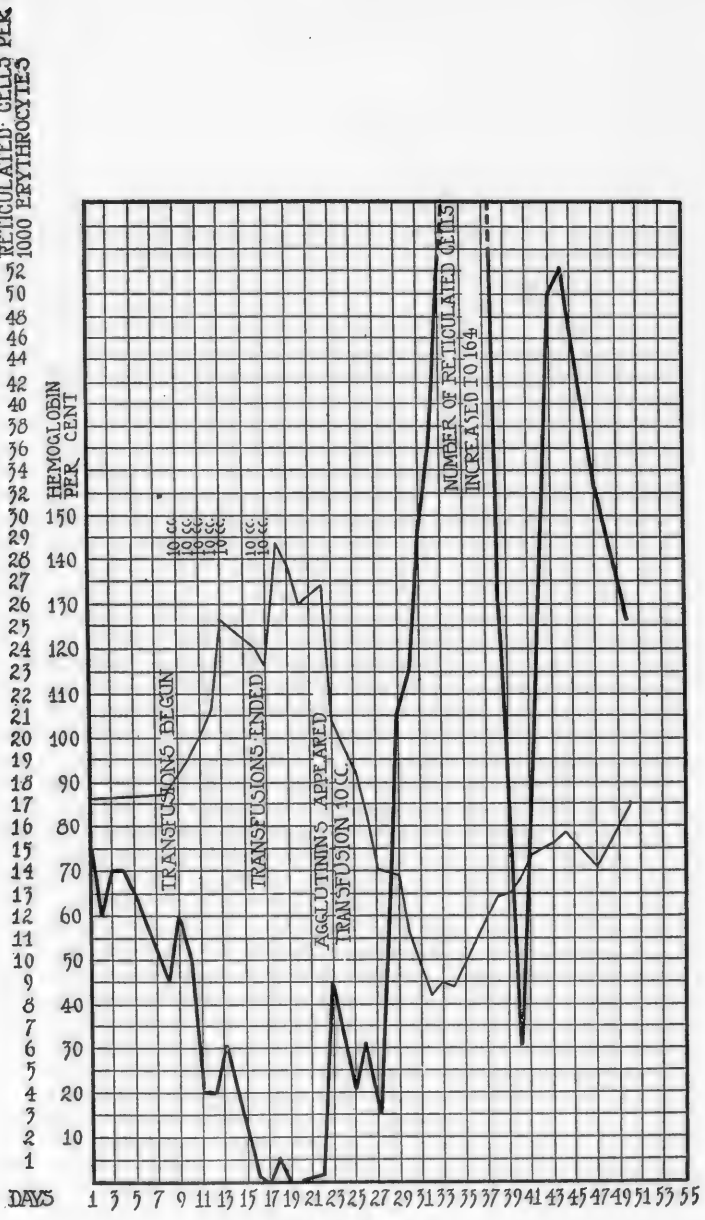
The anomalous behavior of one rabbit, whose chart is shown in Text-fig. 3, strongly supports this explanation of the phenomenon. In this animal there came at length a drop in the hemoglobin, but instead of progressing to an anemia, as in the three rabbits described above, it sank to a point only slightly below normal. The failure of anemia to develop here would seem to be due to the fact that during the plethora, blood production had been maintained at almost its normal rate as indicated by the lack of any but a slight diminution in the number of circulating reticulated cells.

The recovery from the anemia in the three rabbits just mentioned was exceedingly rapid. A similar rapid recovery was noted by Muir and M'Nee[9] and Itami,[10] following experimental hemolytic anemias.

[9] Muir, R., and M'Nee, J. W., *J. Path. and Bacteriol.*, 1911–12, xvi, 410.
[10] Itami, *Arch. exp. Path. u. Pharm.*, 1910, lxii, 104.


RETICULATED CELLS PER 1000 ERYTHROCYTES
HEMOGLOBIN PER CENT
DAYS
TRANSFUSIONS BEGUN
TRANSFUSIONS ENDED
AGGLUTININS PRESENT
RETICULATED CELLS INCREASE TO 336 PER 1000
RETICULATED CELLS INCREASE TO 99 PER 1000
RETICULATED CELLS AT 62 PER 1000
10 cc.
15 cc.

TEXT-FIG. 4.


RETICULATED CELLS PER 1000 ERYTHROCYTES
HEMOGLOBIN PER CENT
DAYS
TRANSFUSIONS BEGUN
TRANSFUSIONS ENDED
AGGLUTININS APPEARED
TRANSFUSION 10 CC.
NUMBER OF RETICULATED CELLS INCREASED TO 164
10 cc.
10 cc.
10 cc.
10 cc.
10 cc.
10 cc.

TEXT-FIG. 5.

Increased Bone Marrow Activity During Subsidence of the Plethora.

The beginning hemoglobin drop in those rabbits which developed an anemia during or after transfusion was marked in each case by a prompt rise in the number of reticulated cells. This increase was rapid, and in two cases the number had risen above normal by the time the hemoglobin had descended to its original level (Text-fig. 4). Then, as the hemoglobin fell further and anemia developed, the reticulated cells continued to increase with great rapidity. The largest number was observed during the early period of regeneration. One rabbit showed a maximum of 594 per 1,000 at this time. They gradually diminished as the animal recovered from the anemia but were still increased when the hemoglobin reached normal again and remained fairly numerous for some time.

Attention is called to the fact that the number of reticulated cells began to increase soon after the hemoglobin started to fall from the plethora level and long before it had reached normal. A further study of this phenomenon was considered worth while. It seemed not unlikely that stimulation of the bone marrow at this time might be brought about by the greatly increased quantity of destroyed blood present. Accordingly an attempt was made to determine this possibility by injecting rabbits intravenously with laked blood. The rabbits were first rendered anemic[11] by bleeding, and the injections of blood were made at different stages of recovery. Some of the rabbits were treated when very anemic; others had practically reached normal before treatment was begun. In order to simulate as nearly as possible conditions of blood destruction occurring *in vivo*, the laked blood was injected at 1 hour or 2 hour intervals for periods of 1 to 3 days. Relatively large quantities were given without apparent ill effect. At no time during the course of the experiment was any increase in the number of reticulated cells noted, nor was other evidence obtained of increased bone marrow activity.

It was then found that simple blood removal from a plethoric animal by bleeding was sufficient to cause a marked bone marrow

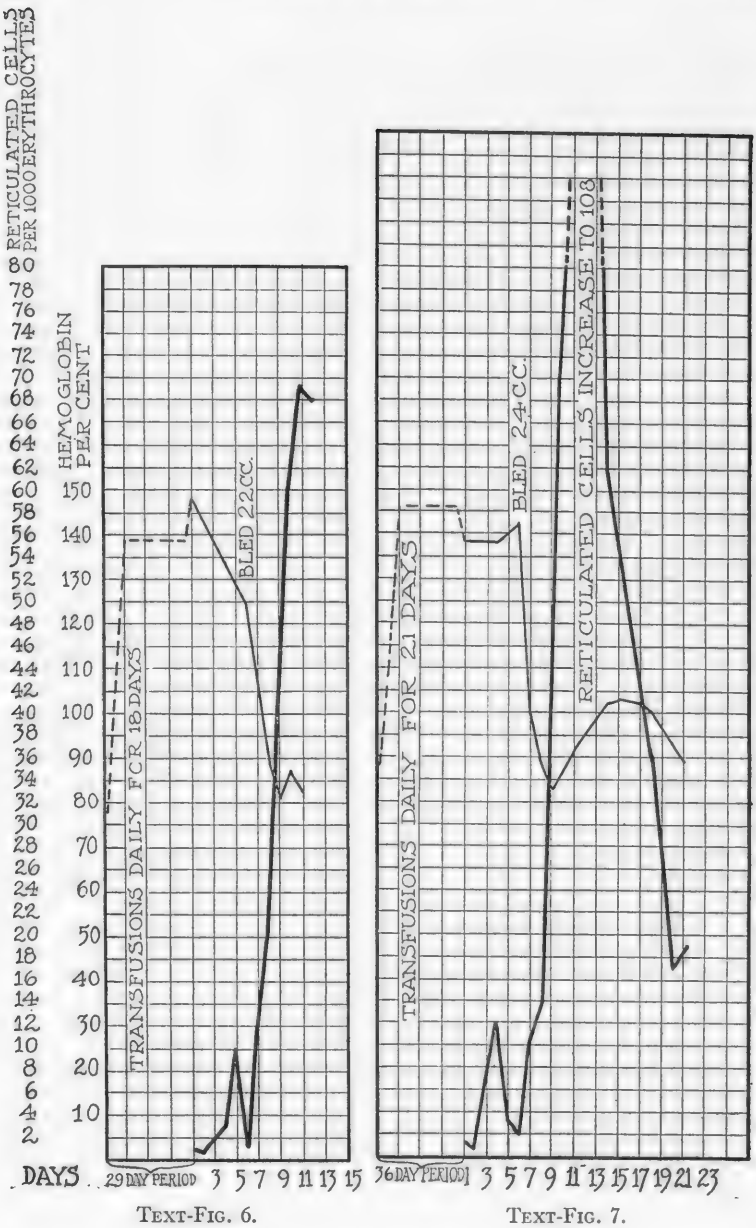
[11] The reason for producing a preliminary anemia in these rabbits is that the experiment was performed originally in an attempt to explain on experimental grounds the cause of the remission in pernicious anemia.

stimulation. Rabbits were made plethoric in the usual way and kept in this condition until the reticulated cells were much diminished. They were then bled a quantity calculated to bring their hemoglobin per cent back to almost normal. An immediate rise in the number of reticulated cells resulted. The normal number, it will be recalled, is 5 to 20 per 1,000 red cells. In one animal treated as above described, they increased to 69 per 1,000 (Text-fig. 6); in another to 80. A third rabbit showed even a greater increase, 108 per 1,000 (Text-fig. 7), but unfortunately the hemoglobin in this case fell after bleeding to slightly below normal. With a fourth animal, the increase was definite, but less marked. In Text-fig. 6 it will be noted that the fall in hemoglobin following bleeding extended over a period of several days, and that the reticulated cells had increased markedly some time before the hemoglobin reached its normal level. Text-fig. 7 shows an instance in which the drop in hemoglobin as a result of bleeding was later followed by a rise above normal. This secondary rise in hemoglobin and the high reticulated cell count were maintained for some days.

Although these experiments are not sufficiently complete to permit one to draw definite conclusions, yet the results would suggest that the increased bone marrow activity accompanying the initial drop of hemoglobin in the plethoric rabbits is due to some functional disturbance of the circulation, resulting from the rapid removal of the plethora. It seems not improbable that this may be a temporary relative oxygen deficiency explainable on the basis that during the period of plethora the organism had in some way adapted itself to a blood of greater oxygen-carrying power. Certainly the blood loss may be thought of as having resulted in a relative anemia.

Color Index.

Observations on the color index in those plethoric rabbits developing anemia revealed striking changes. The normal color index for rabbits is 0.65 to 0.75. During regeneration it was greatly increased, usually to 1.0 or even to 1.08. This finding is of interest particularly in its relation to the accompanying greatly increased percentage of reticulated cells in the blood. It would indicate that reticulation is



TEXT-FIG. 6. TEXT-FIG. 7.

not necessarily associated with a deficiency in the hemoglobin content of the cells and that this association in the blood of secondary anemia is largely fortuitous. In two of the present instances, the reticulated cells numbered 34 per cent and 60 per cent respectively of the total number of red cells, yet the color index was increased 50 per cent over the normal. Even allowing for the somewhat increased size of the reticulated cells, it does not seem possible that they contained less hemoglobin per unit of cell substance than the accompanying more normal red cells. In fresh films they were observed to be notably well colored.

Transfused Reticulated Cells.

In the work as thus far discussed, a possible source of error has not been considered; namely, that of the reticulated cells introduced with the transfused blood. The number of these may, and indeed sometimes must be, considerable, because the bone marrow of the animals furnishing the blood has been stimulated by repeated bleeding. After many transfusions one might expect the recipients to show high counts of reticulated cells. As a matter of fact the reverse is the case. What becomes of the reticulated cells introduced, whether they perhaps mature into non-reticulated corpuscles or instead are destroyed, remains to be determined. As bearing on the point, it should be mentioned that the microcytes of secondary anemia due to hemorrhage are largely derived from fragmentation of reticulated cells, and that in plethoric animals the fragmentation of red blood cells is much increased.[7]

No observations were made on the effect of plethora on the number of white cells or platelets.

Clinical Bearing.

The effect of experimental plethora on the bone marrow of rabbits has a direct bearing on certain unfavorable results which may occur after transfusions in human beings. Clinicians have observed that some cases of pernicious anemia receiving transfusion show no stimulation, but instead unmistakable signs of bone marrow depression.

Vogel and McCurdy[6] were the first to report a systematic study of the reticulated cells in pernicious anemia patients with transfusion. In several of their cases, a decided drop in the per cent of reticulated cells occurred following transfusion. They attributed this to the diluting effect of the newly introduced blood, which had a much lower content of reticulated cells. These patients did badly.

At the Massachusetts General Hospital, Minot and Lee[12] have recently observed the effect of transfusion on the per cent of reticulated cells in a number of cases of pernicious anemia. In certain of their patients, a very marked diminution in the reticulated cells occurred following transfusion. This was accompanied by a leukopenia, a reduction in the number of platelets, and in some cases purpura as well, all of which went to form the picture of general bone marrow depression. As was to be expected, there was no increase in the red corpuscles after transfusion in these patients, other than that referable to the alien blood introduced. In their cases, the amount of blood transfused did not exceed 600 cc. and in one case it was only 300 cc. No such effects as those described above were observed after transfusions of less than 300 cc.

From the results described in the present paper, one may draw tentative conclusions as regards such instances. In pernicious, as in any form of anemia, oxygen deficiency resulting from blood loss doubtless constitutes the stimulus for increased blood production. It is conceivable that in severe conditions, a stage is reached where the bone marrow becomes so exhausted that there is danger of its failure to respond any longer to this stimulus; in other words, the stimulus of oxygen deficiency has grown relatively less effective and may at any time become insufficient. The introduction of a large quantity of blood into the circulation has inevitably the effect of reducing oxygen lack. The sudden lowering of stimulus thus brought about may result in a diminished activity of the bone marrow. The inference is clear that in pernicious anemia with a sluggish bone marrow as shown by the count of reticulated cells, small transfusions are preferable to large ones.

[12] Dr. Minot and Dr. Lee have very kindly allowed me to use these data, which they have not yet published.

SUMMARY.

With the purpose of determining whether a diminished activity of the bone marrow could be brought about experimentally, plethora was produced in rabbits by means of repeated small transfusions of blood. Counts of the number of reticulated red cells in the circulating blood were made during the course of the experiments as an index to changes in the activity of the bone marrow.

With the development of plethora, the number of reticulated cells in the blood decreased. In the majority of the plethoric animals, this diminution was extreme, and in some instances, reticulated cells practically disappeared from the blood. A comparison of the red bone marrow of these animals with that of normal controls revealed a marked reduction in the content of reticulated cells.

After a number of transfusions, there occurred in some of the plethoric rabbits a sudden and marked drop in hemoglobin. The hemoglobin continued to fall until a severe grade of anemia was reached. This was followed by an extremely rapid regeneration accompanied by a striking rise in color index. During regeneration, the reticulated cells were enormously increased in number.

Taken together, these facts show that the bone marrow is markedly influenced by plethora. The diminished number of reticulated cells observed, both in the circulating blood and in the marrow, would make it appear that a decided decrease in blood production occurs. The reduction in the number of these cells cannot be due to changes in the constitution of the red cells put out by the bone marrow, as a result of an increased quantity of hemoglobin in the body, because during regeneration from the above mentioned anemia, when the color index was very high, reticulated cells were still present in large numbers. That the activity of the bone marrow does actually diminish during plethora is further evidenced by the occurrence of the anemia. The most reasonable explanation of this phenomenon is that the recipient develops an immunity against the blood of the donors, which results in the destruction of the strange cells that are in circulation. In keeping with this conception is the appearance of isoagglutinins for the donors' red cells in the blood of the recipient, at about the time of the beginning fall in hemoglobin. The occurrence of anemia as a result of the destruction of the alien blood only would seem to be

due to the circumstance that, during the period of plethora, blood production is greatly diminished; as a consequence, the blood cells proper to the recipient are gradually reduced in number and replaced by alien cells until the latter come to constitute the bulk of the animal's blood.

In those rabbits developing anemia, the initial drop of hemoglobin from the plethoric level to the normal was constantly accompanied by a marked rise in the number of reticulated cells. This brought up a subsidiary problem for study. With the idea that the stimulation of the bone marrow might be due to the presence of an increased quantity of broken down blood, rabbits were injected intravenously with large amounts of laked blood cells. The procedure had no evident effect on the blood picture. It was then found that simple blood removal from a plethoric animal which brought back the hemoglobin to the normal level, or even to a point somewhat above, sufficed to cause a marked increase in the number of reticulated cells. Although these findings are not conclusive, they suggest an explanation for the increased bone marrow activity accompanying the initial drop of hemoglobin in the plethoric rabbits; namely, that the organism had in some way adapted itself during the period of plethora to the presence of a greater amount of blood and that the result of blood loss in such an organism was a relative but not absolute anemia.

The finding that the activity of the bone marrow can be depressed by the introduction of a large quantity of blood into the circulation accounts for the diminished bone marrow activity which sometimes occurs after transfusion in pernicious anemia. In such cases there is a marked drop in the number of reticulated cells and other evidence of bone marrow depression; the patient shows no benefit from transfusion or may grow rapidly worse. The cause of this depression is best explained on the basis that in severe instances of the disease where exhaustion of the bone marrow is imminent, the stimulus of the anemia is only just sufficient to keep the marrow functioning. A sudden lowering of this stimulus is brought about by the introduction of a large quantity of blood into the circulation, and the result is a fall in the activity of the bone marrow. It follows from this that in pernicious anemia with a feebly reacting bone marrow as indicated by the number of reticulated red cells, small transfusions are preferable to large ones.

EXPERIMENTAL STUDY OF ORGANIZATION IN LOBAR PNEUMONIA.

By B. S. KLINE, M.D.

(*From the Pathological Laboratory of the Montefiore Home and Hospital, New York.*)

Plates 17 to 19.

(Received for publication, March 30, 1917.)

Healing of injured and intensely inflamed vascular tissues is characterized by the formation of granulation tissue. A striking exception to this is the perfect healing of the lung in most cases of lobar pneumonia. Here, complete resolution with removal of the exudate and regeneration of the injured lung result. Occasionally, however, the exudate persists, and, as elsewhere, it is dealt with by an ingrowth of capillaries and connective tissue cells.

The factors determining the result in the inflamed lung are little understood and the explanations offered for the occurrence of unresolved lobar pneumonia are as inconclusive as those for the perfect healing of the lung. Ziegler (1) suggested that the retention of the exudate and its organization in unresolved lobar pneumonia probably depended upon recurring exudates, eventually requiring organization for their removal. Von Kahlden (2) considered an excessive formation and retention of fibrin the probable cause. Kohn (3) believed that organization of the exudate occurred as a result of irritation of the interlobular and pleural connective tissue. Köster (4) thought that blocking of the lymph channels in the involved area was responsible. Marchand (5) considered alcoholism a predisposing factor, and Heschl (6) reported its greater frequency in emaciated individuals. Amburger (7) advocated syphilis as the cause. Another conception was that of Corrigan (8) who described it as a special disease analogous to cirrhosis of the liver.

In 1903 Flexner (9) found that consolidated lung in the stage of red hepatization autolyzed imperfectly, whereas in the stage of gray hepatization it autolyzed rapidly and perfectly. He concluded that in unresolved pneumonia because of some disproportion between the leukocytes and other constituents, or other cause unknown, the inflammatory exudate failed to autolyze perfectly, could not be absorbed, and hence underwent organization.

A few years later Opie (10) in the course of a study of leukocytic enzymes noted that when a mixture of dog serum, leukocytes, and fibrin were incubated, autolysis of the leukocytes and digestion of the fibrin were inhibited, whereas when no serum was present, the leukocytes autolyzed readily and the fibrin was completely digested.

In 1914, in association with Winternitz (11), a study of the circulation in the pneumonic lung was made. It was observed that in the later stages of lobar pneumonia, the circulation through the consolidated portion is extremely poor. This was strikingly shown in animals stained with trypan blue. Two sets of experiments were undertaken. In one series, rabbits were injected intravenously with trypan blue, and, following the injection of the dye, the animals were given an intrabronchial injection of pneumococci. The trypan blue was again introduced intravenously during the progress of the pneumonia. In the other series of animals, the first procedure was the injection of pneumococci intrabronchially, and 40 or more hours later the intravenous injections of trypan blue were begun. In the animals receiving trypan blue first, and during the course of the pneumonia, the consolidated portions of the lung at autopsy were intensely blue, whereas in those animals in which the trypan blue was injected only in the later stages of the pneumonia, it was found that but very little of the dye penetrated the consolidated portions. It was inferred that little or no serum reaches the exudate in the alveoli in the stage of gray hepatization, and it was suggested that if this were true, autolysis of the leukocytes and digestion of fibrin in the lung in the late stages of pneumonia are not inhibited and resolution therefore occurs.

Not only do the experiments mentioned above suggest that the absence of the serum from the exudate may be a determining factor in bringing about resolution, but they likewise suggest that the persistence of the exudate in unresolved pneumonia may depend upon the presence in the exudate of sufficient serum to prevent the autolysis of the leukocytes and digestion of the fibrin. If this is true, persistence of the exudate and its organization, in lobar pneumonia, might be expected to develop in those cases in which the circulation in the lung, in the later stages of the process, was good enough to permit a considerable amount of serum to reach the exudate in the alveoli.

The experiments to be reported in this paper were undertaken to determine the significance of the presence of serum in the exudate, in the late stages of pneumonia. The method of injecting the serum depended upon an observation made in the study mentioned above (11). It was found that although very little of the trypan blue,

when injected intravenously, penetrated the lung in the later stage of pneumonia, the exudate offered no barrier to its penetration when it was injected intrabronchially.

The experiments were carried out in dogs because of this animal's resistance to the pneumococcus. Twenty-five animals in all were injected. In nineteen lobar pneumonia was produced with a Group I pneumococcus, according to the technique of Lamar and Meltzer (12). In each case, before the injection of the pneumococci, an x-ray was taken to show the position of the catheter. Seven of the animals were kept as controls, without further treatment. In the other twelve, sterile dog blood serum was injected into the same lobe that received the pneumococci once a day for 4 to 7 days, beginning 48 to 72 hours after the intrabronchial injection of pneumococci. On each occasion x-rays were taken to make sure of the proper position of the catheter.

In addition to the nineteen animals mentioned above, six normal dogs were injected intrabronchially, once a day for 4 to 7 days, with corresponding amounts of sterile dog blood serum.

All the animals were killed in from 9 to 18 days after the first intrabronchial injection. The results are shown in Tables I to III.

TABLE I.

Dog No.	Pneumococci per kilo intrabronchially.	Serum per kilo intrabronchially. Daily, 4 to 7 injections.	Length of life after pneumococcus injections.	Lesion organization.		Lung culture.
				In 1/6 of lobe or over.	Few small areas.	
	cc.	*cc.*	*days*			
1	2.5	2.5	9	+		Sterile.
2	3	3	18	+		"
3	4	4	18	+		"
4	3.5	3.5	17	+		"
5	3.5	3.5	17	+		83 colonies of Gram-negative bacilli.
6	3	3	15		+	
7	3.5	3.5	17		+	
8	3.5	3.5	17		+	
9	2.8	2.8	15	−	−	
10	2.5	2.5	15	−	−	
11	3.5	3.5	17	−	−	
12	3.3	3.3	18	−	−	

TABLE II.

Dog No.	Pneumococci per kilo intrabronchially.	Length of life after pneumococcus injections.	Lesion organization.	
			In 1/6 of lobe or over.	Few small areas.
	cc.	*days*		
13	3.8	18	—	—
14	2.2	15	—	—
15	3.5	17	—	—
16	3.5	17	—	—
17	4.3	18	—	+
18	2.25	9	—	+
19	4	15	—	+

TABLE III.

Dog No.	Serum per kilo intrabronchially. Daily, 4 to 7 injections.	Length of life after first injection of serum.	Lesion organization.	
			In 1/6 of lobe or over.	Few small areas.
	cc.	*days*		
20	4.6	15	—	—
21	3.3	15	—	—
22	4.16	13	—	—
23	4.5	13	—	—
24	3.5	15	—	—
25	3.5	15	—	—

ILLUSTRATIVE PROTOCOL.

Dog 1.—Weight 12,000 gm.

May 22, 1916, 9.20 p.m. Catheter 17 F inserted into bronchus. X-ray taken (catheter in bronchus to right lower lobe). 30 cc. of 29 hour culture of Group I pneumococcus in broth injected through catheter.

May 25–30. 30 cc. of sterile dog blood serum injected daily through catheter in right lower lobe. X-rays taken before each injection.

May 31, 4 p.m. Animal etherized.

Autopsy.—Performed at once. Right lower lobe much more voluminous than normal. Large, solid masses palpable. On section numerous small and larger solid, heavy, airless, consolidated areas are made out, involving one-half of lobe. One area in center the size of small walnut; one near periphery the size of a large walnut. Other areas smaller. These solid patches have a smooth surface and have a grayish red appearance. In branches of the bronchus of this lobe there are firm grayish white plugs, in some places partially, in others completely occluding

the lumen. Histological examination shows the majority of the alveoli in the consolidated portion partially filled with wandering cells (large mononuclear and polymorphonuclear), varying amounts of fibrin, and numerous fibroblasts, in many places associated with fresh capillaries. The process is strikingly shown in the bronchioles where the fibroblasts and fresh capillaries invading the retained exudate are very prominent.

Film preparations of the lung culture taken at the time of autopsy (five loops of expressed tissue juice) on blood agar showed no growth at the end of 3 days.

It will be seen from Table I that in the majority of animals receiving repeated injections of the serum into the pneumonic lung, complete resolution did not occur. In most of these, 9 to 18 days after the production of pneumonia, areas of consolidation in one-sixth or over of a lobe, were present, and microscopically the exudate in the bronchioles and alveoli of the consolidated portions showed extensive organization. In several of the animals the areas of organizing pneumonia were smaller. (Figs. 1 to 5.)

Cultures taken from the lung, in which areas of consolidation were present in one-sixth or more of a lobe, showed no growth in four of the five cases. In the fifth a number of colonies of a Gram-negative bacillus were obtained.

In the majority of dogs with pneumonia not treated with serum, complete resolution occurred (Table II). In two of the three animals in which complete resolution did not occur, no macroscopic areas were observed at the time of autopsy, but a study of the sections showed a small number of alveoli containing exudate undergoing organization. In the third animal there were about twelve pin-head to lentil-sized areas. Most of these showed microscopically an exudate of polymorphonuclear leukocytes, mononuclear cells, scattered red blood cells, and little fibrin. The areas showing organization of the exudate were no more extensive than in the two mentioned above.

In the animals receiving repeated doses of serum alone (Table III) no areas of consolidation were found at autopsy, and microscopically the lungs showed no exudate of any kind.

CONCLUSION.

The experiments reported above give evidence that in unresolved lobar pneumonia, the persistence of the exudate, followed by organization, depends upon the presence of serum in the exudate.

I wish to acknowledge my indebtedness to Dr. T. Scholz, of the X-ray Department, for his cooperation, and to Dr. W. G. MacCallum, for helpful suggestions.

BIBLIOGRAPHY.

1. Ziegler, E., Lehrbuch der speciellen pathologischen Anatomie, Jena, 11th edition, 1906, 786.
2. von Kahlden, C., *Zentr. allg. Path. u. path. Anat.*, 1897, viii, 561.
3. Kohn, H. N., *Münch. med. Woch.*, 1893, xl, 42.
4. Köster, K., *Berl. klin. Woch.*, 1883, xx, 748.
5. Marchand, F., *Virchows Arch. path. Anat.*, 1880, lxxxii, 317.
6. Heschl, R., *Vrtljsschr. prakt. Heilk.*, 1857, liv, 21.
7. Amburger, G., *Deutsch. Arch. klin. Med.*, 1883, xxxiii, 508.
8. Corrigan, *Jahrb. ges. Med.*, 1838, xx, 297.
9. Flexner, S., *Univ. Penn. Med. Bull.*, 1903–04, xvi, 185.
10. Opie, E. L., *J. Exp. Med.*, 1905, vii, 316.
11. Kline, B. S., and Winternitz, M. C., *J. Exp. Med.*, 1915, xxi, 311.
12. Lamar, R. V., and Meltzer, S. J., *J. Exp. Med.*, 1912, xv, 133.

EXPLANATION OF PLATES.

PLATE 17.

FIG. 1. Bronchiole showing persistent exudate undergoing organization, with attachment to the wall in three places. Dog 2, intrabronchial injection of pneumococci, followed by five daily injections of serum. Killed 18 days after onset of pneumonia. Magnification about 260.

FIG. 2. Alveoli and bronchioles containing persistent exudate undergoing organization. Dog 3, intrabronchial injection of pneumococci, followed by five daily injections of serum. Killed 18 days after onset of pneumonia. Magnification about 260.

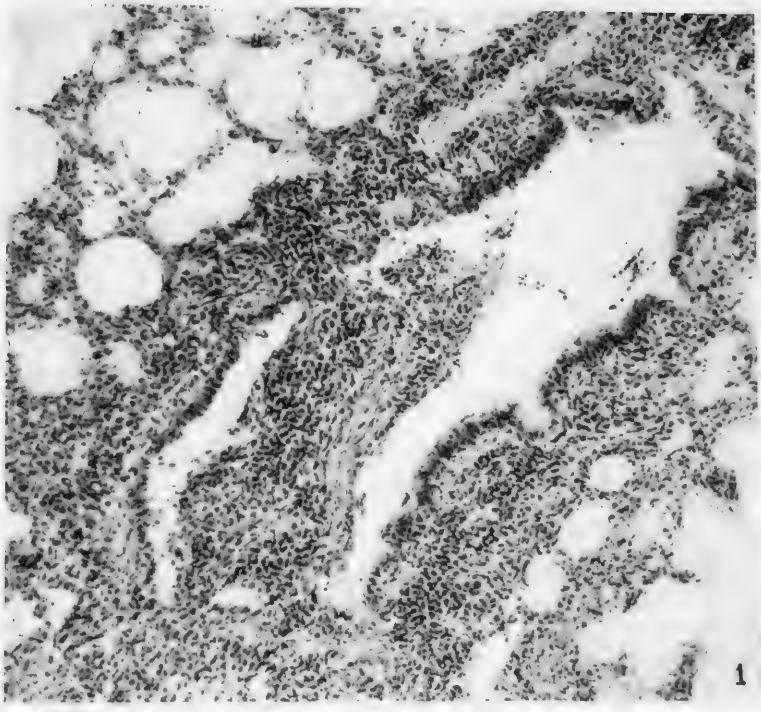
PLATE 18.

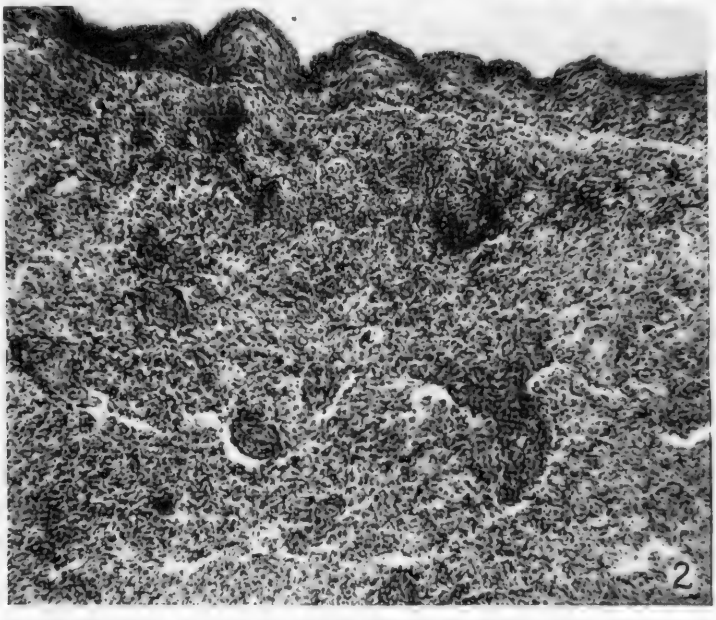
FIG. 3. Alveoli and bronchioles containing persistent exudate undergoing organization, showing extension of process through the pores of Kohn. Dog 3. Magnification about 260.

FIG. 4. High power of portion of the same section as Fig. 1 (Dog 2), showing attachment of exudate to bronchiole wall and ingrowth of fibroblasts. Magnification about 800.

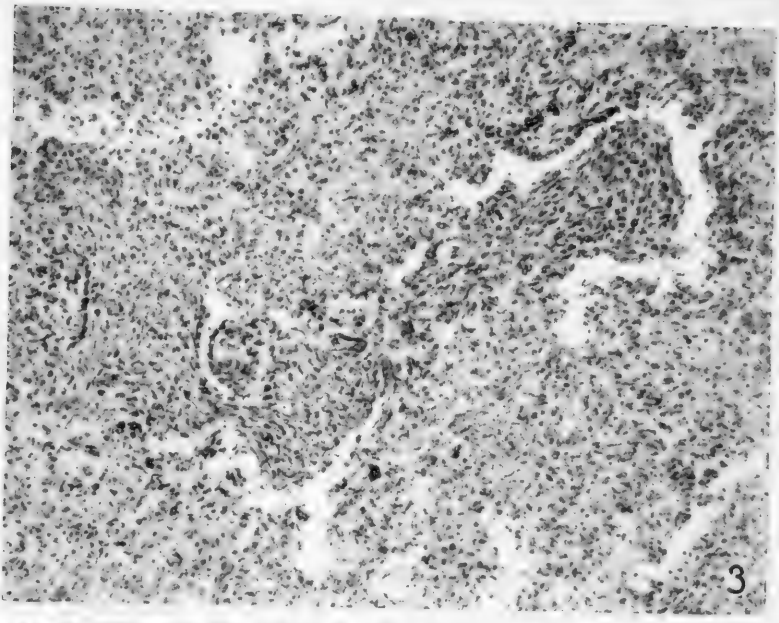
PLATE 19.

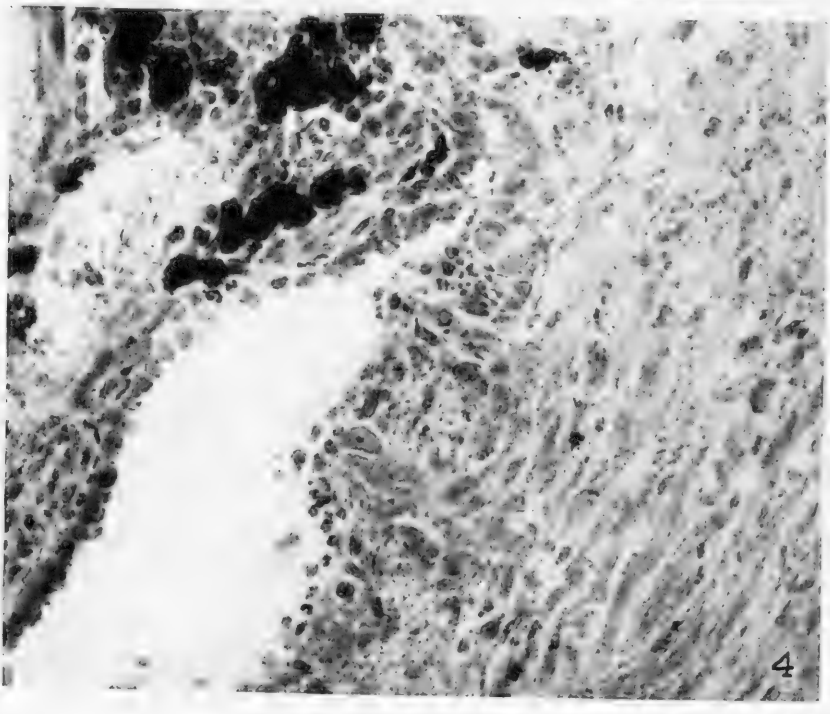
FIG. 5. Another portion of same section as Fig. 1 (Dog 2), showing attachment of exudate to wall and ingrowth of fibroblasts and capillary. Magnification about 800.



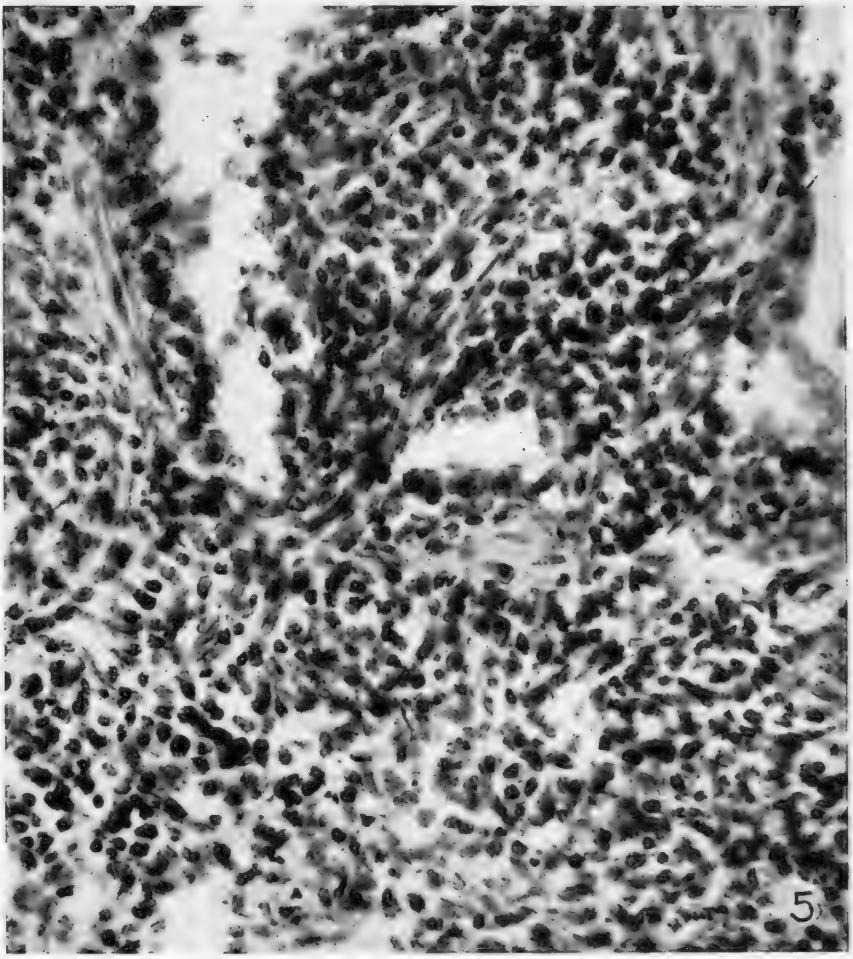


(Kline: Organization in lobar pneumonia.)





(Kline: Organization in lobar pneumonia.)



(Kline: Organization in lobar pneumonia.)

THE GROWTH OF THE OVARIAN FOLLICLE OF THE GUINEA PIG UNDER NORMAL AND PATHOLOGICAL CONDITIONS.

By L. S. N. WALSH, M.D.

(From the Department of Comparative Pathology of Washington University Medical School, St. Louis.)

(Received for publication, April 2, 1917.)

As the ovary seems to be a favorable organ for the quantitative estimation of cell growth, we undertook to analyze the growth energy of the granulosa cells in the ovaries of normal guinea pigs and also to study the growth energy of these cells under certain pathological conditions.

Method.

The growth energy was estimated by the determination of the percentage of mitoses in granulosa cells in the follicles of the ovaries of normal guinea pigs. A large number of cells was counted and the number of mitoses ascertained, thus making possible a comparison of the proliferative power of the various follicles by the relative percentage of mitoses.

The follicles were classified according to size into three groups, large, medium, and small, whose average measurements were: large, 788 by 611 μ; medium, 506 by 450 μ; small, 215 by 130 μ. These measurements are from the inner border of the theca interna on one side to the same point on the other side; *i.e.*, the space lined by the theca interna.

One other type of follicle was studied in normal ovaries; *viz.*, the mature type whose characteristics have been described in earlier papers by Loeb.[1] These follicles are on an average somewhat larger than the ordinary large follicle. There is relatively much more

[1] Loeb, L., *J. Morphol.*, 1911, xxii, 37; *Virchows Arch. path. Anat.*, 1911, ccvi, 278; *Zentr. Physiol.*, 1910–11, xxiv, 203.

cytoplasm in the cells which in hematoxylin- and eosin-stained sections is pink and gives the follicle a much pinker appearance than others in the same ovary.

Measurements of large, medium, and small follicles follow and are of the space lined by the theca interna.

Large Follicles.

Long diameter, between 1,076 and 550 μ.
Short " " 830 " 377 μ.
Average long diameter, 788 μ.
" short " 611 μ.

Diameter of Follicular Cavity.

Long diameter, between 973 and 434 μ.
Short " " 726 " 256 μ.
Average long diameter, 747 μ.
" short " 502 μ.

Medium Follicles.

Long diameter, between 650 and 408 μ.
Short " " 615 " 315 μ.
Average long diameter, 506 μ.
" short " 450 μ.

Diameter of Follicular Cavity.

Long diameter, between 575 and 169 μ.
Short " " 457 " 92 μ.
Average long diameter, 347 μ.
" short " 101 μ.

Small Follicles.

Long diameter, between 318 and 77 μ.
Short " " 227 " 77 μ.
Average long diameter, 215 μ.
" short " 130 μ.

Diameter of Follicular Cavity.

Long diameter, between 188 and 23 μ.
Short " " 108 " 29 μ.
Average long diameter, 80 μ.
" short " 69 μ.

Several of each of the preceding types of follicles, usually three, were studied in each ovary. In order to count the cells and mitoses we studied the follicle in serial sections with a $\frac{1}{12}$ oil immersion objective and a No. 8 ocular upon whose distal surface two parallel lines were ruled. This method presents some difficulties, one constant source of error being the confusion which results from focusing on the cells at different levels. Such errors are uniform throughout and although they depreciate the value of the estimation of the absolute number of cells in a follicle of a given size to some extent, their influence is practically nil from a comparative standpoint, the error being the same in all cases.

Growth of the Normal Follicle.

The protocol of Guinea Pig 1 gives the results obtained in the three sizes of follicles approximately the same as the average figures based on all the follicles studied (Table I). The two ovaries of the animal are numbered 1 and 2. It is shown here that there is a distinct difference in the percentage of mitoses of the different types of follicles. The medium follicles have the highest percentage of mitoses and therefore the greatest growth energy, the large ones the lowest, and the small follicles have a proliferative power which slightly exceeds that of the large type, but is considerably less than that of the medium follicle. This fact holds good in either ovary alone as well as the combined figure of both ovaries.

Table II shows the injurious effect of extirpation of corpora lutea by burning and the effect of slight postmortem change on the percentage of mitoses.

This difference in the growth energy of the various types of follicles is constant, whether we consider the follicle of one or two ovaries of the same animal, if the number of cells counted is sufficiently large, and furthermore, approximately the same relative difference in the percentage of mitoses was seen in all the normal ovaries examined.

Small follicles have relatively more variability in the percentage of mitoses than medium or large follicles. Various sized follicles and mature follicles studied in the different ovaries have been grouped and tabulated separately.

Table II gives a summary of the number of granulosa cells and mitoses counted with the percentage of mitoses in the large follicles of the ovaries of two normal guinea pigs, and also the ovaries of two guinea pigs in which the follicles were abnormal.

TABLE I.

Protocol of Guinea Pig 1.

Follicles in Ovary 1.				Follicles in Ovary 2.			
Follicle.	No. of cells.	No. of mitoses.	Percentage of mitoses.	Follicle.	No. of cells.	No. of mitoses.	Percentage of mitoses.
Large follicles.							
			per cent				*per cent*
No. 1	2,009	19	0.94	No. 1	2,604	8	0.30
" 2	3,550	14	0.39	" 2	3,125	21	0.67
" 3	2,390	25	1.0	" 3	2,349	23	0.97
" 4	2,122	28	1.3				
" 5	2,294	19	0.83				
Total.....	12,365	105	0.84	Total....	8,078	52	0.64
Medium follicles.							
No. 1	1,431	19	1.3	No. 1	2,617	36	1.3
" 2	2,685	20	0.74	" 2	2,076	25	1.2
" 3	2,437	27	1.1	" 3	2,173	15	0.68
Total.....	6,553	66	1.0	Total....	6,866	76	1.1
Small follicles.							
No. 1	749	5	0.66	No. 1	846	3	0.35
" 2	492	5	1.0	" 2	1,441	17	1.1
" 3	681	7	1.0	" 3	747	4	0.53
Total.....	1,922	17	0.88	Total....	3,034	24	0.78

Large Follicles.—In Table II the figures for four different guinea pigs are given. The figures given for Guinea Pigs 1 and 2 are compiled from a study of normal follicles and represent the true growth energy of large follicles, whereas the percentage of mitoses in the granulosa cells of Guinea Pigs 3 and 4 has been lowered in one case by burning out of the corpora lutea and in the other by postmortem change.

To summarize, the growth energy of eighteen large follicles has been studied. In normal ovaries 23,954 cells have been counted having a percentage of mitoses of 0.73. In abnormal ovaries 25,344 cells have been counted with a percentage of mitoses of 0.16.

TABLE II.

Percentage of Mitoses in Large Follicles.

Guinea pig No.	Ovary.	No. of cells.	No. of mitoses.	Percentage of mitoses.	Remarks.
					Normal follicles.
				per cent	
2		3,511	19	0.54	Heat 21 days before death.
1	1	12,365	105	0.84	7 days after ovulation.
	2	8,078	52	0.64	
Total.....		23,954	176	0.67* 0.73†	
					Pathological follicles.
3		8,417	12	0.14	Corpora lutea burned out of ovary.
4	1	8,813	0	0.0	Found dead. Ovaries taken out sev-
	2	8,114	29	0.35	eral hours after death.
Total.....		25,344	41	0.16* 0.16†	

* This figure represents the average obtained from the percentage of the respective ovaries.

† This figure represents the percentage of mitoses for the total of all the cells counted in that particular type of follicle, the total being compiled from the figures of all the follicles counted in the various animals.

Medium Follicles.—The figures of medium follicles are shown in Table III. The percentage of mitoses in all these follicles is 1 or more, with the exception of Guinea Pig 3 which is the animal from whose ovaries the corpora lutea had been burned out. Doubtless the low percentage of mitoses here is due to the heating of the ovary in the process of burning out the corpora lutea.

To summarize, in normal medium follicles (Guinea Pig 3 is excluded) 80,625 cells have been counted, having a percentage of mitoses of 1.2.

TABLE III.

Percentage of Mitoses in Medium Follicles.

Guinea pig No.	Ovary.	No. of cells.	No. of mitoses.	Percentage of mitoses.	Remarks.
				per cent	
5	1	13,924	147	1.0	10 days after birth of young.
	2	10,990	160	1.4	
6	1	5,823	76	1.3	Near the end of pregnancy.
	2	6,771	96	1.4	
7	1	4,828	55	1.1	Ovulation had just taken place.
	2	5,086	60	1.1	
2	1	9,428	128	1.3	Heat 21 days before removal of ovaries.
	2	10,356	131	1.2	
1	1	6,553	66	1.0	7 days after ovulation.
	2	6,866	76	1.1	
3		4,464	21	0.47	Corpora lutea burned out of ovary.
Total.....		80,625	995	1.1* 1.2†	Total does not include Guinea Pig 3.

* Average percentage of mitoses.

† Absolute percentage of mitoses.

Small Follicles.—Table IV shows that there is relatively greater variability in the percentage of mitoses of small, than either large or medium follicles. The growth energy of small follicles in various stages of cavity formation was compared, but no constant differences were found. Here again the ovaries of Guinea Pig 4, in which there has been slight postmortem change, showed a very low percentage of mitoses and their figures together with those of Guinea Pig 3, from whose ovaries the corpora lutea were burned out, are not included in the total.

To summarize, in normal small follicles 89,545 cells have been counted, having a percentage of mitoses of 0.66.

Mature Follicles.—The growth energy of six mature follicles in the ovaries of two guinea pigs was studied (Table V). In all, 24,445

TABLE IV.

Percentage of Mitoses in Small Follicles.

Guinea pig No.	Ovary.	No. of cells.	No. of mitoses.	Percentage of mitoses.	Remarks.
				per cent	
5	1	49,520	305	0.61	10 days after parturition.
	2	10,241	25	0.24	
6	1	6,693	84	1.20	Near the end of pregnancy.
	2	1,713	12	0.70	
2	1	6,084	22	0.36	Heat last observed 21 days before death.
	2	2,432	13	0.53	
7	1	3,831	60	1.56	Ovulation had just taken place.
	2	4,075	30	0.73	
1	1	1,922	17	0.88	7 days after ovulation.
	2	3,034	24	0.78	
3		3,929	28	0.71	Corpora lutea burned out of ovary.
4	1	3,754	3	0.0	Ovaries taken out several hours after death.
	2	6,473	17	0.26	
Total.....		89,545	592	0.75* 0.66†	Total does not include Guinea Pigs 3 and 4.
Total of Guinea Pigs 3 and 4.....		14,156	48	0.33	

* Average percentage of mitoses.
† Absolute percentage of mitoses.

TABLE V.

Percentage of Mitoses in Mature Follicles.

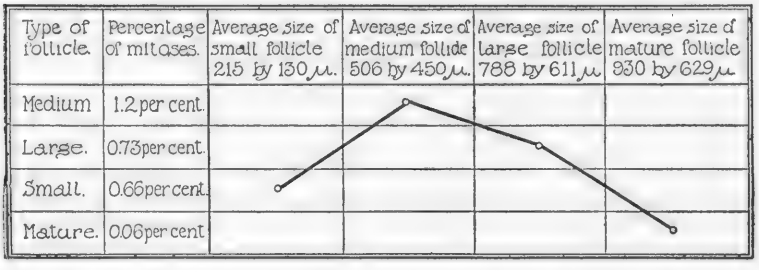
Guinea pig No.	Ovary.	No. of cells.	No. of mitoses.	Percentage of mitoses.
				per cent
6		15,640	5	0.03
8	1	3,418	8	0.23
	2	5,387	4	0.07
Total.....		24,445	17	0.11* 0.06†

* Average percentage of mitoses.
† Absolute percentage of mitoses.

cells and 17 mitoses were counted with an average percentage of mitoses for the follicles of the respective ovaries of 0.11, whereas the absolute number of mitoses per hundred cells is 0.06.

It follows, therefore, that mitoses are almost absent in mature follicles, which agrees with the observation of Loeb[1] and others that mitoses are very infrequent in mature follicles.

Growth Curve.—If we represent the variation in the growth energy in accordance with the development of the different follicles, we obtain a curve such as is represented in Text-fig. 1. We see that the growth energy increases with the development of the follicle from small to medium size, and here it reaches its maximum, then it again


Type of follicle.
Percentage of mitoses.
Average size of small follicle 215 by 130 μ.
Average size of medium follicle 506 by 450 μ.
Average size of large follicle 788 by 611 μ.
Average size of mature follicle 930 by 629 μ.
Medium
1.2 per cent.
Large.
0.73 per cent.
Small.
0.66 per cent.
Mature.
0.06 per cent.

TEXT-FIG 1. Curve indicating the proliferative energy of follicles as a function of their size.

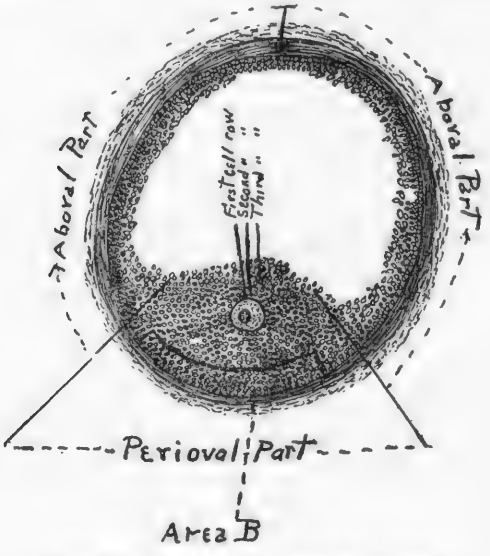
decreases, the growth energy of large follicles being at approximately the same level as that of the small follicles. A steep fall to mature follicles than takes place.

This curve bears some resemblance to that described by Robertson[2] and Ostwald[3] and some others for various growth processes. However, Robertson and Ostwald based their results not on percentage increases but on absolute increases. If the curves of Robertson and Ostwald were expressed in percentage increases they would differ markedly from ours. As Loeb has pointed out, it is probable that the decrease in growth energy of mature follicles is associated with greater cell differentiation in these follicles.

[2] Robertson, T. B., *Arch. Entwcklngsmechn. Organ.*, 1908, xxv, 581.

[3] Ostwald, W., in Roux, W., Vorträge über Entwickelungsmechanik der Organismen, Heft 5, Leipsic, 1908.

Quantitative Determination of the Location of Mitoses in the Granulosa Cells of Follicles of Various Sizes.—It has been observed by Loeb that the mitoses occurring in mature follicles are found in the discus proligerus. This observation suggested the possibility that the ovum stimulated the proliferation of the granulosa cells and made it desirable to investigate more closely the localization of mitoses in various kinds of follicles.



TEXT-FIG. 2. Areas in the ovarian follicle.

A number of follicles in the various ovaries having only a few granulosa cells surrounding the egg were selected. The number of mitoses occurring in the various cell rows around the egg was determined and, in addition, whenever a cavity had been formed the part of the granulosa in which the mitoses occurred was noted. Moreover, measured areas at various distances from the egg were compared as to the frequency of their mitoses.

In order to facilitate the description of our findings we found it necessary to divide the granulosa into several parts (Text-fig. 2). We divide the granulosa into two main parts: the perioval part which comprises the discus proligerus and the few cells distal to it which

are indicated on the diagram; the remaining part of the granulosa we call the aboval part. We also distinguish within the perioval part an area designated B on the diagram, of which the length is variable, but never exceeds the limitations of the perioval part, and the short diameter extends from the theca interna behind the egg towards it to the point where the cells begin to be more loosely arranged. We determined the relative frequency of mitoses in the peri- and aboval parts and in addition compared the number of mitoses per sq. μ in area B with the aboval part.[4]

26 small follicles in which the egg was surrounded by from five to ten rows of granulosa cells, were analyzed in the manner just described, with the following result. 8 of the 26 follicles (30 per cent) have 100 per cent of their total mitoses occurring in the first cell row. 17, including the above 8 (65 per cent), have 50 per cent or more of their total mitoses occurring in the first cell row. 4 (15 per cent) have less than 50 per cent of their total mitoses occurring in the first cell row, but more mitoses near the egg than near the theca interna. 3 (11 per cent) have their greatest number of mitoses midway between the egg and the theca. 2 (7 per cent) have more of their total mitoses near the theca than near the egg.

It has been shown here that 65 per cent of the total mitoses occur in the first two cell rows, 30 per cent of which are in the first cell row. By total mitoses we understand all the mitoses observed by study of the follicle in serial section. By first cell row we mean the row of cells encircling the egg (Text-fig. 2).

Four small follicles, in which the space lined by the theca interna had an average measurement of 253 by 170 μ and in which there was early cavity formation, were studied. The findings were as follows:

1st	cell	row	contains	16	per	cent	of	the	total	mitoses	of	the follicles.
2nd	"	"	"	5	"	"	"	"	"	"	"	"
3rd	"	"	"	10	"	"	"	"	"	"	"	"
4th	"	"	"	8	"	"	"	"	"	"	"	"
5th	"	"	"	6	"	"	"	"	"	"	"	"
6th	"	"	"	6	"	"	"	"	"	"	"	"
7th	"	"	"	0.8	"	"	"	"	"	"	"	"
Remaining part of granulosa contains				44	"	"	"	"	"	"	"	"

[4] Because of the variability of length of area B sometimes a few cells of the perioval part were included in the aboval part.

It is seen here that as the follicle increases in size the percentage of the total mitoses which occur in the first and second cell rows, decreases, yet 30 per cent of the mitoses still occur in the first three cell rows. The proliferative power of the cells near the theca is greater than in follicles of smaller size.

Next the results obtained in the study of nine small follicles, the cavities of which are of somewhat larger size than the preceding two kinds, are given. The average diameter of the space lined by the theca interna in these follicles was 215 by 132 μ; the average size of the cavity 72 by 71 μ.

1st	cell	row	contains	14	per	cent	of	the	total	mitoses	of	the	follicles.
2nd	"	"	"	9	"	"	"	"	"	"	"	"	"
3rd	"	"	"	8	"	"	"	"	"	"	"	"	"
4th	"	"	"	1	"	"	"	"	"	"	"	"	"
5th	"	"	"	9	"	"	"	"	"	"	"	"	"
6th	"	"	"	3	"	"	"	"	"	"	"	"	"
9th	"	"	"	1	"	"	"	"	"	"	"	"	"
Remaining part of granulosa			contains	52	"	"	"	"	"	"	"	"	"

The results here are similar to those obtained in the preceding follicles. As the follicles increase in size there has been a gradual increase in the number of mitoses not in the immediate neighborhood of the egg.

The results of the analysis of the localization of mitoses in thirteen medium follicles, in which the average diameters of the space lined by the theca interna were 506 by 450 μ, are given below.

1st	cell	row	contains		4	per	cent	of	the	total	mitoses	of	the follicles.
2nd	"	"	"		1	"	"	"	"	"	"	"	"	"
3rd	"	"	"		3	"	"	"	"	"	"	"	"	"
4th	"	"	"		2	"	"	"	"	"	"	"	"	"
5th	"	"	"		4	"	"	"	"	"	"	"	"	"
6th	"	"	"		2	"	"	"	"	"	"	"	"	"
7th	"	"	"	less than	1	"	"	"	"	"	"	"	"	"
Remaining part of granulosa			contains		80	"	"	"	"	"	"	"	"	"

In medium follicles the remaining part of the granulosa includes a considerable number of cells in the perioval part of the follicle,

while in small follicles with a cavity the remaining part of the granulosa is made up almost entirely of cells in the aboval part.

In medium follicles the migration of the proliferating area from the first few cell rows encircling the egg to the cells near the theca behind the egg (see area B in Text-fig. 2), has been completed and now the greatest percentage of the mitoses of the follicle occurs in area B, as we shall see more specifically later on, and hence the majority of the 80 per cent of the mitoses in the remaining part of the granulosa occurs in this area.

The migration of the area of greatest mitotic activity reaches its maximum distance from the egg synchronously with the maximum growth energy of the follicle. Perhaps this is partly accounted for by the fact that the cells are farther apart and their number may be relatively smaller.

The analysis of the localization of mitoses in large follicles brought out the fact that the observation of Loeb that mitoses in mature follicles are limited to the discus proligerus, also to a certain extent holds good in large follicles which are not mature, as is shown by the following data.

Several large follicles were studied in the usual way and it was found that 43 per cent of their total mitoses occurred in the first two cell rows encircling the egg; 50 per cent occurred in the perioval (Text-fig. 2), and 7 per cent in the aboval part. The percentage of the total mitoses has increased in the first two cell rows coincidently with the decrease in growth energy of the follicle.

The difference in the growth energy of the perioval and aboval parts of large follicles is much greater than in medium sized ones, as in the case of the former the proliferative power of the aboval part has come almost to a standstill. This agrees with the observation of Loeb that degenerative processes usually attack the cells of the discus proligerus last.

It seems reasonable to assume on the basis of the preceding data that the egg may furnish a direct stimulus to the granulosa cells, especially in view of the fact, as will be brought out later, that the site of greatest mitotic activity is always near the egg.

We next compared the frequency of mitoses in the peri- and aboval parts of the follicles. We also compared the number of mitoses in the

aboval part reduced to the sq. μ equivalent of area B, with the latter, with the following results.

20 Small Follicles.

Perioval part contains 72 per cent of the total mitoses.
Aboval " " 26 " " " " " " "
Area B contains 80 per cent of the total mitoses.
Aboval part, reduced to sq. μ equivalent of area B, contains 19 per cent of the total mitoses.

6 Medium Follicles.

Perioval part contains 66 per cent of the total mitoses.
Aboval " " 33 " " " " " " "
Area B contains 71 per cent of the total mitoses.
Aboval part, reduced to sq. μ equivalent of area B, contains 25 per cent of the total mitoses.

2 Large Follicles.

Perioval part contains 93 per cent of the total mitoses.
Aboval " " 7 " " " " " " "
Area B contains 85 per cent of the total mitoses.
Aboval part, reduced to sq. μ equivalent of area B, contains 14 per cent of the total mitoses.

To compare area B with the aboval area, we determined the number of sq. μ in each and multiplied the number of mitoses counted in area B by the number of times the aboval part exceeded area B in sq. μ.

As a result of our study of the localization of mitoses in the granulosa cells we draw the following conclusions: (1) In all follicles the large majority of mitoses occurs in the perioval part. (2) In medium follicles where the percentage of mitoses is relatively highest, there are more mitoses in area B and in the aboval part than in large or small follicles. Moreover, in medium follicles there is a relative preponderance of cells some distance from the egg as compared with large and small, where they are more directly around the egg, showing that with increase in the percentage of mitoses in medium follicles, the granulosa cells not directly around the egg, gain relatively more in proliferative power than the cells around the egg.

Growth of the Follicle under Pathological Conditions.

We analyzed the proliferative power of follicles under two pathological conditions: (*a*) In follicles in which part of the granulosa cells had undergone degeneration by karyorrhexis, a condition which corresponds to early follicular degeneration; (*b*) secondly we investigated the condition in the follicles of hypotypical ovaries.

(*a*) *Growth Energy of Follicles in Beginning of Degeneration of Granulosa Cells.*—Only follicles which showed rather extensive degeneration by karyorrhexis were considered. In this form of atresia the degeneration is usually most evident in the cell rows nearest the cavity of the follicles. The growth energy in these follicles was determined in the same way as in normal ones and only those cells were counted in which there was no visible degeneration. The follicles and their cavities were usually somewhat larger than medium follicles. The results of the examination of twenty-seven follicles with partial granulosa degeneration are given in Table VI.

TABLE VI.

Percentage of Mitoses in Degenerating Follicles.

Guinea pig No.	Ovary.	No. of cells.	No. of mitoses.	Percentage of mitoses.	Remarks.
				per cent	
5	1	6,789	14	0.20	
	2	8,310	45	0.54	
6	1	7,247	10	0.13	
	2	2,304	0	0.0	
2	1	6,450	29	0.44	
	2	7,143	50	0.69	
3		2,604	0	0.0	Corpora lutea burned out.
4	1	4,870	0	0.0	Found dead.
	2	4,036	1	0.02	
Total.....		49,753	149	0.22*	
				0.29†	

* Average percentage of mitoses.

† Absolute percentage of mitoses.

It is shown here that both the absolute and average percentage of mitoses of the cells in these follicles which are not yet degenerating are much lower than in normal follicles of the same size.

It was observed during this study that the majority of the mitoses occurs in the cells of the discus proligerus and that the percentage of mitoses is proportionate to the amount of degeneration; the more extensive the degeneration the weaker is the proliferative power of the cells which have not yet degenerated.

(*b*) *Growth Energy of Follicles of Hypotypical Ovaries.*—Under this designation Loeb[5] has described ovaries which he has observed under several conditions, for instance, after the burning out of corpora lutea, etc. Hypotypical ovaries are smaller than normal; they are characterized by an early connective tissue atresia of follicles, taking place while the follicles are quite small, and hence there are no large, no mature follicles, and for the time being the ovary is sterile. It was our intention to determine if possible whether the failure of follicles in such ovaries to grow past small size was due to a diminished proliferative power of the granulosa or to an increased cell destruction. We may mention here that granulosa degeneration by karyorrhexis does not take place in the hypotypical ovaries except in isolated cells and not *en masse* as in normal follicles.

The results obtained in fifty-five follicles, in six hypotypical ovaries of three animals, are presented in Table VII. As has already been mentioned, the follicles in hypotypical ovaries become atretic at so early a stage of their development that only small follicles are seen well preserved. The average diameters of the space lined by the theca interna, of the follicles whose figures appear in Table VII, are as follows.

Long diameter of space lined by theca interna from 277 to 131 μ; average 209 μ.
Short " " " " " " " " 246 " 211 μ; " 178 μ.
Long " " cavity from 188 to 35 μ; average 91 μ.
Short " " " " 208 " 54 μ; " 135 μ.

Table VII shows that the average percentage of mitoses (0.57) and the absolute percentage (0.55) in the hypotypical follicles are nearly the same, but slightly lower than in normal follicles of the same size.

In the granulosa cells of the hypotypical follicles, those cells which remain preserved show almost the same proliferative power as the

[5] Loeb, *Zentr. Physiol.*, 1911–12, xxv, 342.

TABLE VII.

Percentage of Mitoses in Follicles of Hypotypical Ovaries.

Guinea pig No.	Ovary.	No. of cells.	No. of mitoses.	Percentage of mitoses.
				per cent
9	1	5,661	12	0.21
	2	5,668	24	0.42
10	1	9,491	51	0.53
	2	2,463	27	1.0
11	1	10,099	57	0.56
	2	8,703	61	0.70
Total.....		42,085	232	0.57*
				0.55†

* Average percentage of mitoses.

† Absolute percentage of mitoses.

granulosa cells of normal follicles. But it would be incorrect to state that the granulosa as such in hypotypical follicles shows the same proliferative power. Evidently a considerable number of cells which remain preserved in normal follicles undergo premature solution in hypotypical follicles and these cells no longer proliferate. Therefore the absolute number of mitoses in follicles of hypotypical ovaries is of course diminished. It is, however, of interest that the cells which remain show only slightly less proliferative power than the cells of normal follicles.

We also made comparative studies of normal and hypotypical follicles. (*a*) We compared the localization of mitoses in the two kinds of follicles and found that the localization phenomenon in hypotypical follicles parallels that of normal follicles of the same size. (*b*) We compared the size of the nuclei in normal and hypotypical follicles by measuring them at various phases in the granulosa in a number of follicles of each kind. It was found that on the whole the nuclei of normal follicles slightly exceeded in size the hypotypical. (*c*) The relative sizes of the cavities of normal and hypotypical follicles were compared and we found that the cavity of the hypotypical is relatively about twice as large as that of the normal follicle of the same size.

SUMMARY AND CONCLUSIONS.

1. The growth energy of the granulosa cells in the ovarian follicles of normal guinea pig ovaries varies with the size of the follicle, and follows a definite growth curve. The growth energy in small follicles is relatively low, then a gradual rise takes place with increase in size of the follicle up to a maximum which is reached when the follicle attains medium size. This ascending part of the curve is followed by a gradual fall in growth energy until in large follicles the proliferative power has fallen almost to the level of small follicles. Synchronously with maturity of the follicles there is an abrupt fall in growth energy to near the zero point.

2. There is a distinct localization of mitoses in the follicle. The large majority of mitoses is found in the perioval part. With increase in the growth energy which characterizes medium follicles, there is an increase in the proliferative power of the cells more distal to the egg, but still near the egg. We may therefore conclude on the basis of our observations and on the previous observations of Loeb, that the egg exerts a stimulus, which causes proliferation of the granulosa cells.

3. Under pathological conditions certain changes occur in the growth energy of the follicle: (*a*) Granulosa degeneration causes in the living cells a diminution in the proliferative power which is proportionate to the extent of the degeneration. (*b*) Hypotypical follicles have approximately the same or only slightly diminished proliferative power as normal ones, and thus their failure to grow is essentially due to disintegration of the granulosa cells.

THE RELATION OF APICAL TUBERCULOSIS OF ADULTS TO THE FOCAL TUBERCULOSIS OF CHILDREN.

By EUGENE L. OPIE, M.D.

(*From the Department of Pathology of Washington University Medical School, St. Louis.*)

PLATES 20 AND 21.

(Received for publication, April 2, 1917.)

In a previous article[1] I have shown that the lungs of almost every individual who has reached adult life (18 years) contain foci of tuberculosis which, occurring in any part of the lung and associated with tuberculosis of the regional lymphatic nodes, have the characters of pulmonary tuberculosis in childhood. In infancy tuberculosis is almost invariably fatal, but between the ages of 2 and 18 years tuberculous lesions of the lung heal with increasing frequency as age advances. Almost every child is spontaneously inoculated or "vaccinated" with tuberculosis. The early experiments of Koch[2] confirmed by subsequent observers have shown that a first infection with tuberculosis modifies the course of a second infection. Koch's fundamental experiment showed that the injection of tubercle bacilli into the subcutaneous tissue of animals already infected with tuberculosis caused a reaction at the site of infection more severe than that of the first infection, but the lesion did not progress and there was no involvement of the regional lymphatic nodes. Experience acquired by attempted immunization of small animals and by the inoculation of cattle with tubercle bacilli of human type has demonstrated that immunity conferred by a first infection with tubercle bacilli is relative and not absolute, and tends to diminish and perhaps disappear after complete healing of the tuberculous lesions caused by inoculation.

Fatal pulmonary tuberculosis of adults and of older children with few exceptions has its origin in the apices of the lungs and like the second infection of animals, produced by Koch and others, is unaccompanied by caseous lesions of the regional lymphatic nodes. These characters serve to separate adult from infantile tuberculosis. The incidence of focal and apical tuberculosis of the lungs in a series of autopsies on children and adults has already been shown.[3] It

[1] Opie, E. L., *J. Exp. Med.*, 1917, xxv, 855.

[2] Koch, *Deutsch. med. Woch.*, 1891, xvii, 101.

[3] See Tables I and III of the previous article,[1] pp. 857 and 865.

is probable that the percentage of focal infections in adults is greater than that indicated by the tables[3] and closely approximates 100 per cent, for in those instances in which no pulmonary lesion was found tuberculosis of lymphatic nodes in two instances demonstrated only by microscopic examination suggested the probability that corresponding pulmonary lesions had been overlooked. Even if this view is incorrect it may be assumed that tuberculosis of lymphatic nodes or of other organs would exert an influence upon resistance similar to that of primary pulmonary tuberculosis. Nevertheless the identification of a focal pulmonary lesion accompanied by tuberculosis of regional lymphatic nodes in the lungs of an adult is significant, for, as the tables show, it indicates that tuberculosis has been acquired in childhood and presumably antedates an apical lesion with which it may be associated. Orth[4] states that the experience of pathological anatomy contradicts the view that there is in every instance of phthisis an older lesion which has had its origin in childhood, and in most individuals who have died of phthisis he has found no such older lesion.

In view of the character of the material which has been available for study it has been the primary purpose of the present investigation to study the relation of non-lethal apical tuberculosis to the focal tuberculosis of childhood. Incidentally there has been an opportunity to compare healed or encapsulated apical tuberculosis with progressive apical phthisis and to determine as far as possible whether apical tuberculosis has a greater tendency to a fatal issue in those who have escaped infection in childhood.

Apical lesions have been regarded as tuberculous when microscopic examination has demonstrated the presence of recognizable tubercles, when caseation has occurred, or when calcified nodules which represent the site of former caseation are embedded within fibrous tissue. There is little reason for doubting that the fibrous scars which often pucker the apical pleura and usually extend as wedge-shaped masses into the lung substance have their origin in tuberculous infection, but in the absence of recognizable tubercles, caseation, or calcification these lesions have not been designated tuberculous. A healed tuberculous focus consisting of fibrous tissue in which are

[4] Orth, J., Drei Vorträge über Tuberkulose, Berlin, 1913.

calcified nodules may be found at one apex, whereas at the other there is only fibrous induration. There is more doubt concerning the nature of fibrous induration which not infrequently involves the pleura over a circumscribed area at the apex of the lung and does not penetrate into the underlying lung tissue. This pleural induration is not infrequently found at the apex of one lung, whereas there is a fibrous scar at the apex of the other lung, and occasionally with a caseous lesion of one lung the opposite apical pleura is indurated.

Table I shows the frequency with which apical tuberculous lesions have occurred at various ages in autopsies on adults and children.

TABLE I.

Age.	No. of autopsies.	Active apical tuberculosis.		Encapsulated apical tuberculosis.		Total apical pulmonary tuberculosis.
Children.						
yrs.			*per cent*		*per cent*	*per cent*
Under 1	43	—	—	—	—	—
1– 2	16	—	—	—	—	—
2– 5	14	—	—	—	—	—
5–10	11	—	—	—	—	—
10–18	9	1	11.1	—	—	11.1
Adults.						
18–30	6	1	16.7	—	—	16.7
30–50	23	1	4.3	2	8.7	13.0
50–70	15	1	6.7	3	20.0	26.7
70+	6	—	—	3	50.0	50.0

The number of cases in each group is small and the percentages represent only crudely the incidence of the various lesions at various ages. Nevertheless they show that the incidence of encapsulated and healed tuberculosis increases with increasing age whereas there is no similar increase in the incidence of active tuberculosis.

Table II shows the frequency of induration affecting the apical pleura and of fibrous scars at the apices of the lungs.

The table shows that these purely fibrous lesions like healing or healed apical lesions which are demonstrably tuberculous are uncom-

TABLE II.

Age.	No. of autopsies.	Induration of apical pleura.		Scars within apex.	
		Children.			
yrs.			*per cent*		*per cent*
Under 1	43	—	—	—	—
1– 2	16	—	—	—	—
2– 5	14	—	—	—	—
5–10	11	—	—	—	—
10–18	9	—	—	—	—
		Adults.			
18–30	6	1	16.7	1	16.7
30–50	23	4	17.4	4	17.4
50–70	15	6	40.0	5	33.3
70+	6	2	33.3	1	16.7

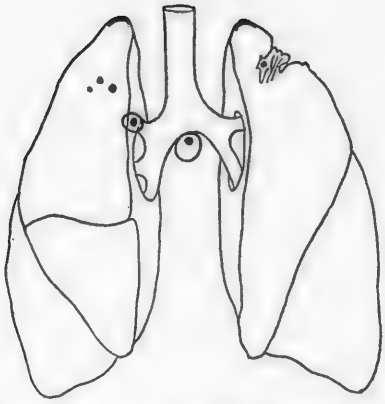
mon in early adult life, but increase in frequency as age progresses. They do not increase after the age of 70 years because at this time pulmonary apices of over half of all individuals are occupied by frankly tuberculous lesions.

Apical pleural induration in all instances has been accompanied by demonstrably tuberculous lesions in some other part of the lungs.

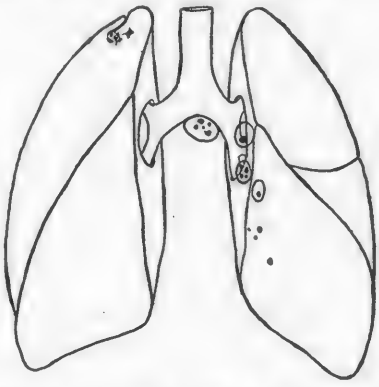
Table I contains eight instances of encapsulated apical tuberculosis. In these lungs focal tuberculous lesions have been found in association with the apical lesions and without doubt have antedated them. The situation of the focal lesion in relation to the apical lesion is shown by Text-figs. 1 to 7[5, 6] and in x-ray plates (Figs. 1, 2, and 3). These focal lesions have been in most instances firmly calcified and completely healed; in five (Text-figs. 1 to 3[5, 6]) of eight instances the focal nodules have been of almost stony hardness. In two instances (Text-figs. 4 and 5) the focal lesions have been caseous and partly calcified (mortar-like), and in one instance (Text-fig. 6) calcified, partially calcified, and encapsulated caseous foci have been found in the lungs.

[5] See also Text-fig. 7 of the previous article.[1]

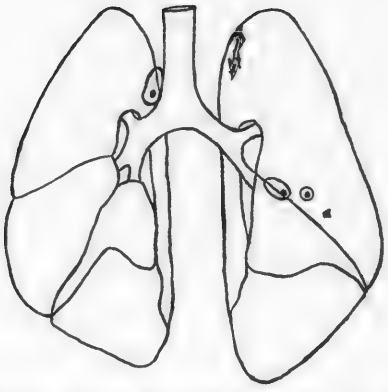
[6] See also Text-fig. 8 of the previous article.[1]



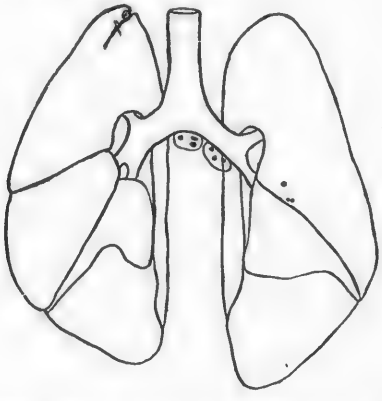
TEXT-FIG. 1. Anterior view of the lungs of a white woman, age 54 years, who died after hysterectomy for carcinoma of the cervix. There are three encapsulated nodules in the upper part of the right upper lobe and calcified nodules are found in lymph glands at the hilus of the right upper lobe and at the bifurcation of the trachea. There is an indurated fibrous area containing a caseous focus situated in contact with the pleura of the external surface of the left upper lobe a short distance below the apex of the lung. The pleura over this area is puckered and at one place drawn far into the substance of the lung.



TEXT-FIG. 2. Posterior view of the lungs of a colored woman, age 60 years, who died of chronic endocarditis, general arteriosclerosis, and cerebral embolism. In the right lower lobe below the pleura of the external surface are three small encapsulated calcified nodules and in the substance of the lobe is a fourth calcified nodule. In the lymph nodes at the hilus of the right lower lobe and at the bifurcation of the trachea are calcified nodules. Just below the apex of the left upper lobe continuous with the indrawn pleura is a thick strand of pigmented fibrous tissue which passes into the substance of the lobe and is continuous with an area of fibrous induration containing two caseous areas.



TEXT-FIG. 3. Median view of the lungs of a white man, age 61 years, who died of hypernephroma of the kidney with metastases to the liver, lymph nodes, and bone, general arteriosclerosis, chronic passive congestion of viscera, ascites, and pleural effusion. There is a calcified nodule in the lower part of the upper left lobe and calcified nodules occur in adjacent lymphatic nodes and in a lymphatic node above the right bronchus. At the apex of the left lung continuous with the pleura of the median surface just below the apex is a narrow mass of fibrous tissue containing a small caseous area.



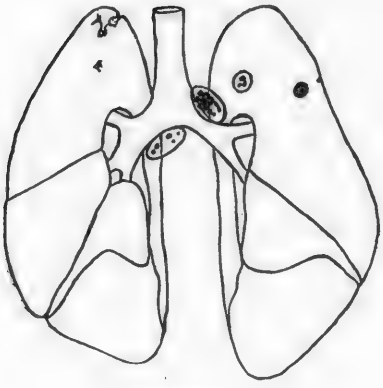
TEXT-FIG. 4. Median view of the lungs of a white man, age 73 years, who died with carcinoma of the sigmoid flexure of the colon, intestinal obstruction, perforation of the intestine and peritonitis, arteriosclerosis, chronic nephritis with granular kidneys, hypertrophy of the heart, and myocarditis. In the lower part of the left upper lobe are three encapsulated partially calcified caseous nodules. In lymph nodes below the left bronchus and at the bifurcation of the trachea are partially calcified caseous nodules. Just below the apex of the right upper lobe extending from the indrawn pleura into the substance of the lung is a coarse strand of fibrous tissue within which is a partially calcified caseous nodule.

The apical lesions have in several instances occupied the greater part of one or both apices and have consisted of numerous caseous tubercles embedded in fibrous tissue pigmented black by inhalation pigment (Figs. 1 and 3 and Text-figs. 6 and 7[5, 6]). In other instances the lesion has consisted of strands or masses of fibrous tissue in which are caseous or caseous and partially calcified nodules (Fig. 2 and Text-figs. 1 to 5). In every instance the lesion is at some point in contact with the pleural surface near the apex of the lung. The pleura at the site of contact with the lesion is puckered and not infrequently deeply drawn into the lesion (Text-figs. 1, 2, and 4).

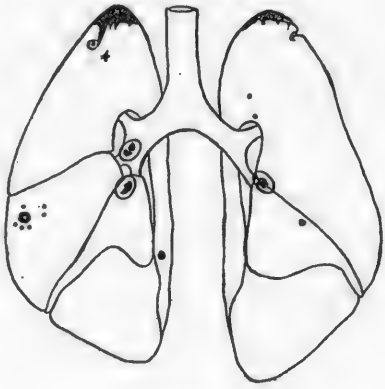
In the instances which have been cited slowly progressive tuberculosis characterized by abundant formation of fibrous tissue has occurred at the apex of the lung and has been associated with focal pulmonary lesions doubtless acquired in childhood. In one instance slowly progressive tuberculosis of organs other than the lung, namely the adrenals, accompanied focal encapsulated caseous lesions of the lower part of the upper lobe of the right lung and encapsulated caseous lesions of the regional lymphatic nodes. Pigmentation of the skin with general weakness had been noted 4 years before death and at autopsy the adrenals were found transformed into masses of dense fibrous tissue containing caseous spots. It is noteworthy that the lesions in this instance were all caseous and encapsulated; beginning calcification of the pulmonary lesion indicated that it was older than those found in other organs.

In one instance (Text-fig. 7) chronic apical tuberculosis in a woman aged 39 years was active and implicated a considerable part of the left lung but found in association with carcinoma of the stomach with metastases in the ovaries, liver, and peritoneum was not the cause of death. In the upper and lower lobes on the right side there were firmly calcified nodules and the regional lymphatic nodes contained similar foci. The presence of abundant pigmented fibrous tissue was index of the slow progress of the lesion. It is not improbable that associated cancer produced conditions favorable to the extension of an apical lesion.

One instance of fatal tuberculosis of the lung complicated by the presence of chronic peritonitis and chronic unilateral pleurisy does not admit of any conclusion concerning the primary seat of infection;



TEXT-FIG. 5.



TEXT-FIG. 6.

TEXT-FIG. 5. Median view of the lungs of a white man, age 40 years, who died of anthracosis of the lungs, hypertrophy of the right heart, and central necrosis of the liver. In the left upper lobe are two caseous partly calcified nodules, the larger 1.2 cm. across; in regional lymphatic nodes within the lung above the right bronchus and at the bifurcation of the trachea are caseous partly calcified nodules. At the apex of the right lung continuous with the puckered pleura is deeply pigmented fibrous tissue containing three small calcified nodules and one caseous area. In the substance of the upper right lobe near the apex is a fibrous area in which are two calcified nodules.

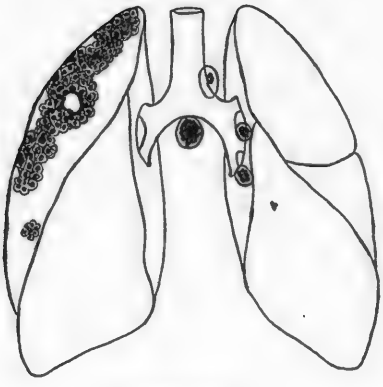
TEXT-FIG. 6. Median view of the lungs of a white man, age 74 years, who died after resection of the cecum for carcinoma with purulent peritonitis, general arteriosclerosis, and chronic nephritis. In the right middle lobe is a calcified nodule 1.2 cm. across, and nearby just below the pleura are a number of small calcified nodules. In the right lower lobe is a partially calcified encapsulated caseous nodule. At the hilus of the right middle and lower lobes are two lymphatic nodes containing encapsulated caseous foci. In the left lower lobe is an encapsulated partially calcified caseous focus, and a similar focus occurs in an adjacent lymph node. Two small calcified nodules occur below the pleura of the median surface of the left upper lobe. The apex of the right upper lobe is occupied by a consolidated fibrous area 1.5 cm. in thickness. It contains several small smooth-walled cavities about 3 mm. in diameter. A fibrous strand extends downward from this indurated area and surrounds a caseous focus. A second caseous focus surrounded by fibrous tissue occurs in the lung substance nearby. The apex of the left upper lobe shows fibrous induration similar to that on the right side forming a zone 0.5 cm. in thickness.

there was a small caseous focal lesion in the upper lobe of the right lung but the lymphatic nodes at the hilus of the lobe contained no tuberculous lesions. This focal lesion was recognized by its shadow 2 mm. across cast upon an x-ray plate and doubtless contained some calcium salts, although there was no macroscopic evidence of calcification. With chronic tuberculous pleurisy there was effusion into the left pleural cavity, atelectasis of the left lung, and chronic tuberculous peritonitis with adhesions firmly matting together the intestines. There was active caseous tuberculosis of the right lung most advanced near the median surface of the organ where the lung had been subjected to the pressure of the effusion in the left pleural cavity. There was some fibrous induration at the apex of the compressed left lung. The tuberculous lesion of the peritoneum and pleura was characterized by the new formation of abundant fibrous tissue and had pursued a chronic course.

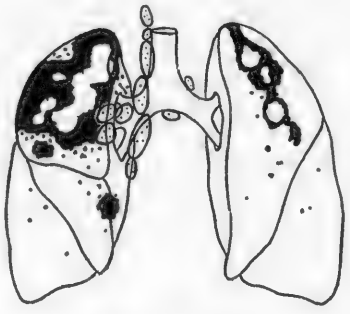
It is noteworthy that in two instances pulmonary tuberculosis has been the cause of death and in neither of these cases were preexisting focal lesions found in the lungs.

Text-fig. 8 is a diagram of tuberculous lesions in the lungs of a child 11 years of age. Almost the entire right upper lobe including the apex is consolidated, in large part caseous, and contains numerous cavities. The upper part of the left upper lobe contains similar consolidated areas in which are cavities. The remainder of the lungs contains scattered tubercles. Lymphatic nodes at the hilus of the right lung and along the right side of the trachea are much enlarged and succulent; there is no evident caseation, but microscopic examination shows the presence of scattered caseous tubercles. The lungs were cut into thin sections and no focal lesions were recognized. There were tuberculosis of the larynx, tuberculous ulcers of the intestine, and tuberculous peritonitis. The von Pirquet tuberculin test was negative 6 weeks before death.

The second instance of fatal tuberculosis (Text-fig. 9) is a remarkable example of acute rapidly progressive disease terminating with tuberculosis of the bodies of the lumbar vertebræ, psoas abscess, tuberculosis of retroperitoneal lymphatic nodes, tuberculosis of the thoracic duct, and disseminated miliary tuberculosis in a man with no old focal tuberculous lesion of the lung. An apical lesion which



TEXT-FIG. 7.



TEXT-FIG. 8.

TEXT-FIG. 7. Posterior view of the lungs of a white woman, age 39 years, who died of carcinoma of the stomach and metastatic carcinoma of the ovaries, liver, and peritoneum. There is chronic pulmonary tuberculosis with abundant formation of fibrous tissue at the upper part of the left upper lobe; cavities have been formed. Several scattered areas of similar consolidation occur in other parts of the lungs; a consolidated partially caseous area in the mid-part of the right lung has been omitted from the diagram. There is an encapsulated calcified nodule 3.5 cm. across in the upper part of the right lower lobe; calcified foci are found in lymphatic nodes at the hilus of the same lobe, at the bifurcation of the trachea, and above the right bronchus.

TEXT-FIG. 8. Anterior view of the lungs of a white child, age 11 years, who died of pulmonary tuberculosis with cavity formation, tuberculous ulceration of the larynx, tubercles in tonsil, intestine, and liver, and tuberculous peritonitis. The von Pirquet tuberculin reaction was negative 6 weeks before death. There is tuberculosis with cavity formation involving both apices but much more advanced on the right side. Lymphatic nodes at the hilus of the right upper lobe, about the right bronchus, and along the right side of the trachea are much enlarged and contain tubercles.

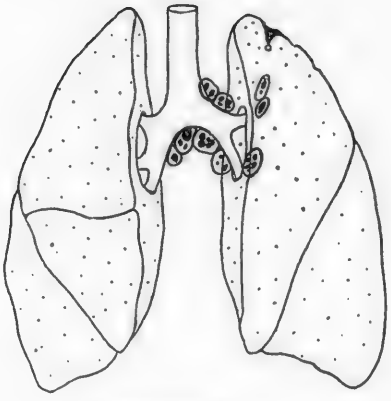
had undergone caseation was found in the left lung immediately below the pleura. In continuity with this lesion was a small dilated bronchus, of which the wall was caseous. Below the level of this caseous bronchus were numerous patches of tuberculous bronchopneumonia. The remainder of both lungs was occupied by numerous miliary tubercles. An x-ray plate (Fig. 4) demonstrates the absence of calcified nodules within the substance of the lung or in the regional lymphatic nodes.

It is particularly significant that in the absence of focal pulmonary lesions lymphatic nodes within the substance of the left upper lobe in proximity to the apical lesion and at the hilus of the lobe are enlarged and caseous (Text-fig. 9). It may be assumed that in the absence of preexisting tuberculosis the apical lesion has caused active tuberculosis of the regional lymphatic nodes. The apical lesion has acted as a first infection.

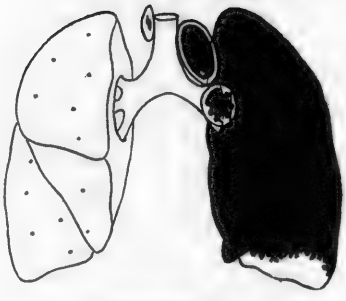
This relation of the regional lymphatic nodes to a first infection with tuberculosis is well illustrated by the spontaneous occurrence of tuberculosis in a macaque monkey kept in confinement. The entire left lung with the exception of a small area at the base is the site of tuberculous pneumonia with widespread caseation (Text-fig. 10). The lymphatic nodes at the hilus of the affected lung are greatly enlarged and partially caseous.

The relation of the focal tuberculous lesions usually acquired in childhood and of the corresponding lesions of regional lymphatic nodes to coexisting apical tuberculosis furnishes no evidence that the apical lesion is derived from the preexisting focal lesion. In most instances the focal pulmonary lesion and the accompanying lesions of lymphatic nodes have been firmly calcified and completely healed whereas the apical lesion is caseous (Text-figs. 1 to 3[5, 6]).

There is little probability that tuberculous infection has been transmitted by way of the lymphatics from the focal lesion of the lung or of the regional lymphatic nodes to the apex. The large lymphatic trunks of the lung fall into two groups; namely, superficial collecting trunks which, situated below the pleura, pass to the lymphatic nodes at the hilus of the lung, and deep collecting trunks which follow the bronchi, the pulmonary arteries, and the pulmonary veins to the nodes at the hilus of the lungs. It is not improbable that



TEXT-FIG. 9.



TEXT-FIG. 10.

TEXT-FIG. 9. Anterior view of the lungs of a colored man, age 28 years, who died of pulmonary tuberculosis, tuberculosis of the body of the second lumbar vertebra, psoas abscess, tuberculosis of retroperitoneal lymphatic nodes, tuberculosis of the thoracic duct, and disseminated miliary tuberculosis. The lungs are studded with miliary tubercles. At the apex of the left lung below the pleura of the external aspect of the lung is a caseous area 2 by 2 cm. on the surface extending in wedge-shaped form 1.5 cm. into the lung. At the apex of the wedge is a dilated bronchus with caseous wall. In the substance of the left upper lobe in a line between the tuberculous lesion in the apex and the hilus of the lung are enlarged partially caseous lymphatic nodes. About the left bronchus and at the bifurcation of the trachea are similar freshly caseous enlarged lymphatic nodes.

TEXT-FIG. 10. Anterior view of the lungs of a macaque monkey which died of tuberculous pneumonia involving almost the entire left lung. Lymphatic nodes at the hilus of the left lung are greatly enlarged and caseous.

lesions of lymphatic trunks or of lymphatic nodes may cause a reversal of the current within lymphatics. In four instances (Text-figs. 1, 2, and 7[6]) focal pulmonary lesions have been situated in the right lung whereas there has been an apical lesion only on the left side. In two instances (Text-fig. 4[5]) with focal lesions in the left lung apical lesions have been found only on the right side. The probability of transmission by lymphatic channels from the lung or lymphatic nodes on the one side to the apex of the opposite lung is scant.

SUMMARY.

The age incidence of focal tuberculous lesions of the lungs demonstrates that they have their origin in most instances in childhood. Focal lesions which heal have been found at all ages after the 2nd year of life, but in more than half of all individuals these lesions are acquired between the ages of 10 and 18 years. In the period between 18 and 30 years at least 85 per cent of all individuals have acquired focal tuberculous lesions. The occurrence of tuberculous infection in the lungs, in regional lymphatic nodes, or in some other organs of the body such as the gastrointestinal tract and its lymphatic system, is nearly universal but doubtless a few individuals escape. That focal tuberculous lesions of the lung are occasionally acquired during adult life is shown by the slight increase in the proportion of those with these lesions as age increases from 18 years to old age.[3]

Apical lesions of the lung make their appearance in later childhood and occur with increasing frequency from adolescence to old age (50 per cent). After the 2nd year of life focal tuberculous lesions occurring in situations other than the apices of the lungs tend to heal and after the 10th year focal lesions are almost invariably encapsulated and latent or healed. Fatal tuberculosis after the 10th year is with few exceptions apical in origin. The apices are not only more susceptible to infection in later life but once infected afford less resistance to the extension of the lesion.

The present series of cases has furnished opportunity to observe the character of the apical lesion in lungs of individuals previously infected with tuberculosis. With one exception the apical lesion (in eight instances) has pursued a chronic course and, encapsulated

by fibrous tissue, has remained limited to the extreme apex of the lung. In one instance in a woman with advanced malignant disease chronic pulmonary tuberculosis has been progressive. Tuberculosis of the apices in those who have previously acquired a focal tuberculous lesion has pursued a chronic course and in most instances has remained latent or has completely healed.

A very small group of instances of fatal pulmonary tuberculosis suggests that apical lesions in those who have not undergone previous infection may assume an unusually severe character. One instance of apical tuberculosis unaccompanied by focal lesions and followed by tuberculosis of the thoracic duct and disseminated miliary tuberculosis has been especially significant. Apical tuberculosis unaccompanied by evidence of preexisting tuberculosis may be accompanied by tuberculosis of the regional lymphatic nodes, whereas apical tuberculosis in an individual with a preexistent focal tuberculous lesion is not followed by tuberculosis of adjacent lymphatic nodes. It is well known that tuberculosis in previously uninfected animals is followed by tuberculosis of adjacent lymphatic nodes, whereas a second infection fails to implicate the regional lymphatic nodes. This relation has been well illustrated by the lungs of a monkey which acquired in confinement acute tuberculous pneumonia limited to the left lung; the lymphatic nodes on this side were greatly enlarged and caseous.

The following observations indicate that apical tuberculosis of adults is not the result of infantile tuberculosis but is caused by subsequent infection: (*a*) Apical tuberculosis does not have its highest incidence, in accordance with common belief, in early adult life when focal infections acquired in childhood are relatively fresh and active but is more common in later life when the focal lesions of childhood have in most instances completely healed. It is noteworthy that most of these apical lesions of later life pursue a chronic course and are discovered at autopsy in individuals who have died from other causes. (*b*) The well characterized lesions of tuberculosis acquired in childhood and found in adults with apical lesions are almost invariably calcified and healed. The apical lesion is in most instances relatively fresh and caseous whereas the focal pulmonary lesion and associated lesions of regional lymphatic nodes exhibit no evidence

of activity. (*c*) In a large proportion of instances of associated focal and apical tuberculosis the focal lesion is in one lung, whereas the apical lesion is limited to the opposite apex. This relation affords no support to the view that tuberculous lesions may be transmitted to the apex by way of the lymphatics.

EXPLANATION OF PLATES.

PLATE 20.

FIG. 1. X-ray plate of the lungs of a man, age 74 years. There is a consolidated area at the left apex containing caseous foci impregnated with enough calcium salts to cast shadows upon the plate. In the right lower lobe and in adjacent lymphatic nodes are completely healed firmly calcified nodules.[7]

FIG. 2. X-ray plate of the lungs of a man, age 40 years. The positions of the right and left lungs are reversed in the plate. At the right apex continuous with the pleura are strands of fibrous tissue in which occur small caseous and calcified areas. In the right upper lobe below the apex is a small fibrous patch containing two caseous partially calcified spots. In the left upper lobe near the inner surface is a firmly calcified encapsulated nodule 1 cm. across. Caseous areas of mortar-like consistency occur in regional lymphatic nodes. No nodule was found, after prolonged search, corresponding to the shadow 2 cm. across in the lower right lobe. Compare with Text-fig. 5 from the same lungs.

PLATE 21.

FIG. 3. X-ray plate of the lungs of a man, age 74 years. The positions of the right and left lungs are reversed in the plate. At the apex of the right lung there is an indurated area containing a caseous spot. The apex of the left lung is indurated. In the right middle and lower lobes, in the left lower lobe, and in regional lymphatic nodes are calcified, partially calcified, or caseous nodules. Compare with Text-fig. 6 from the same lungs.

FIG. 4. X-ray plate of the lungs of a man, age 28 years, who died of acute miliary tuberculosis. There are no shadows suggesting the presence of calcified nodules in the lungs or in their lymphatic nodes. Compare with Text-fig. 9 from the same lungs.

---

[7] Text-fig. 8 of the previous article[1] is a diagram of these lungs.

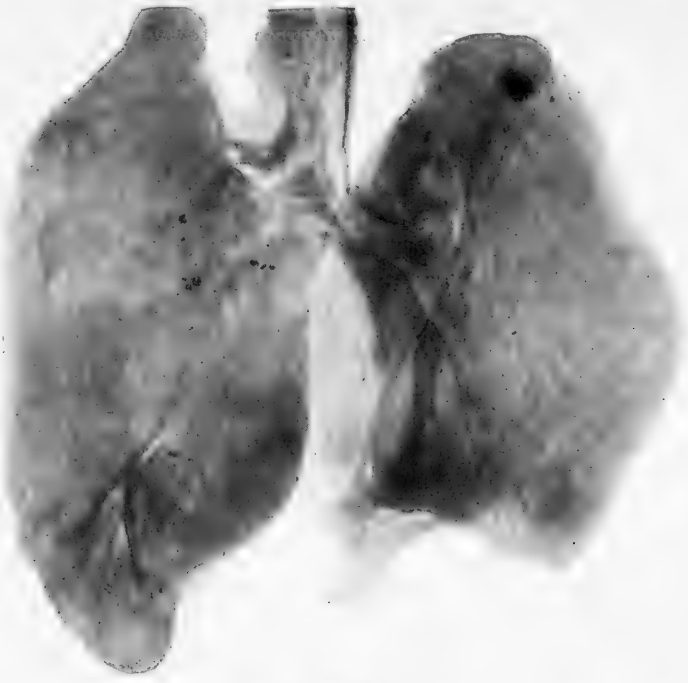


FIG. 1.

FIG. 2.

(Opie: Tuberculosis of adults and children.)

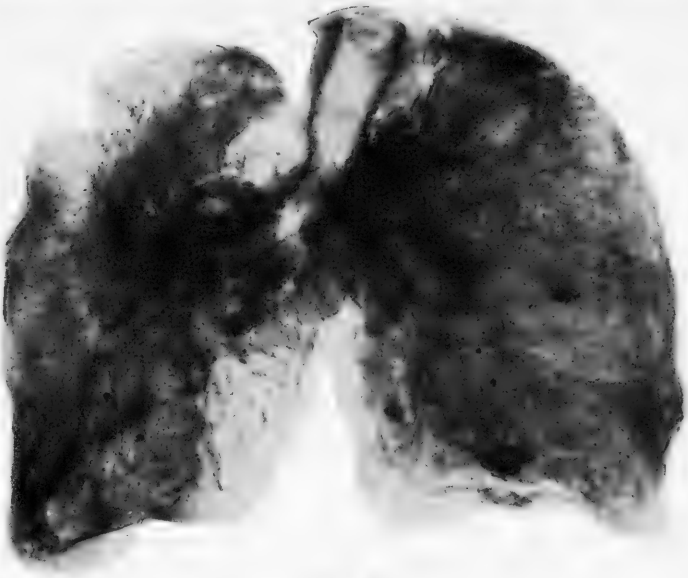


FIG. 3.

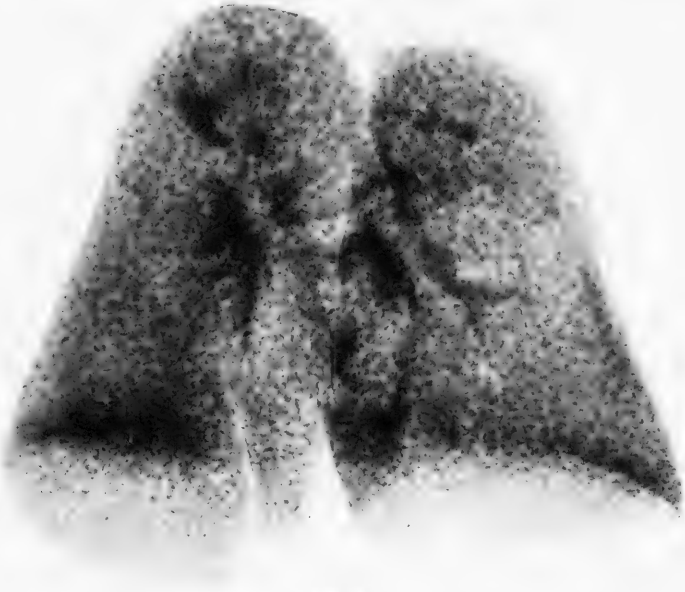


FIG. 4.

(Opie: Tuberculosis of adults and children.)

CICATRIZATION OF WOUNDS.

IX. Influence on the Healing of Wounds of Variations in the Osmotic Tension of the Dressing.

By ALEXIS CARREL, M.D., P. LECOMTE DU NOÜY, and ANNE CARREL.

(From the Laboratories of The Rockefeller Institute for Medical Research, New York, and Hospital 21, Compiègne, France.)

(Received for publication, March 22, 1917.)

In previous articles it has been shown that the curve representing the process of cicatrization of an aseptic wound is geometric,[1, 2] and can be calculated by the formula of du Noüy. The comparison of the calculated and observed curves alters the study of the effect upon cicatrization of a substance applied to the surface of the wound. If the daily decrease of the area of a wound is known, and if the wound is maintained in a condition of surgical asepsis, the modifications in the rate of the healing process can be attributed to the special action of the substance applied, and the extent of this action can be accurately measured.

Up to the present it has not been known to what degree the rate of cicatrization of a wound can be affected by the dressing. Surgeons have studied the influence of so called healing substances when applied to wounds, but no precise conclusion has resulted from these observations. No method existed of measuring exactly the surface of a wound and of calculating with any degree of accuracy the rate of the healing process. On the other hand, the bacteriological condition of the wounds experimented upon was never taken into consideration. Such modifications of the rate of healing as were noticed might be equally attributable to the action of the substance employed upon the bacteria of the wound as to the tissues themselves.

[1] Carrel, A., and Hartmann, A., *J. Exp. Med.*, 1916, xxiv, 429.

[2] du Noüy, P. L., *J. Exp. Med.*, 1916, xxiv, 451.

The following experiments were undertaken to ascertain whether modifications in the osmotic tension of the dressing exert an influence on the rate of repair.

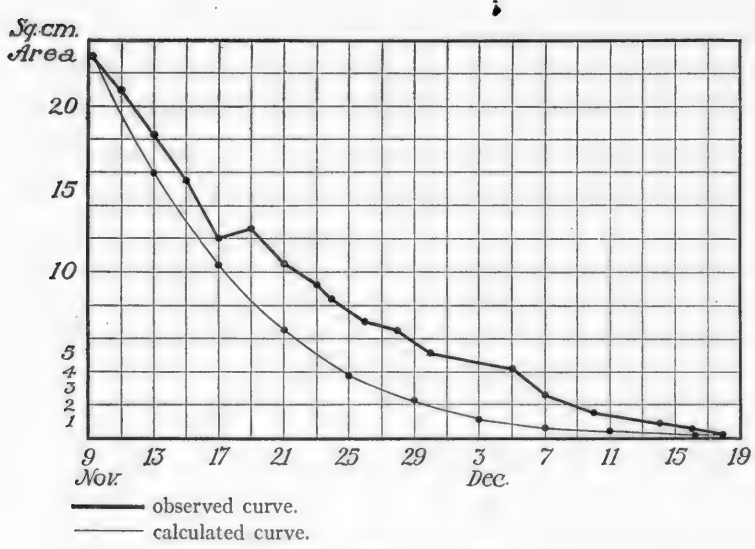
EXPERIMENTAL.

Surface wounds already covered with granulating tissue were selected. The normal rate of cicatrization of the wound was first obtained by sterilization with Dakin's hypochlorite solution or Daufresne's chloramine paste. The experiment was started as soon as the regular progress of the observed curve, as compared with the curve calculated according to du Noüy's formula, was established. The measurements of the wound and the plotting of the curve were made according to the technique previously described. Distilled water or hypertonic solution was applied to the surface of the wound by means of small perforated rubber tubes bound at the extremities and enveloped by a small cylindrical pad of Turkish toweling. The length of the pad varied from 4 to 8 cm., according to the size of the wound. Four threads placed transversely across the pad extended over the wound and were attached to the skin by means of small adhesive patches in such a manner as to keep the flushing tube in a fixed position over the granulations. The sterile liquid was contained in a flask placed about 1 meter above the patient's mattress, and reached the flushing tube by means of a Murphy drop tube. As a rule, in these experiments about 125 gm. of fluid flowed out per hour. Another technique was also used to bring into contact the surface of the wound and the distilled water or hypertonic solution. Agar cakes containing distilled water or hypertonic sodium chloride solution were applied to the surface of the wound. These constantly gave up their fluid contents, and were left at the surface of the wound, according to the nature of the experiment, from 6 to 12 hours and even 24 hours per day. In order to prevent immediate reinfection, the wound was flushed from four to six times in 24 hours with Dakin's solution. Another method consisted in applying for several hours every day a dressing composed of sodium stearate and chloramine paste. Chloramine paste, 10 parts per 1,000 was generally used, because reinfection takes place under 4 parts per 1,000. The wound

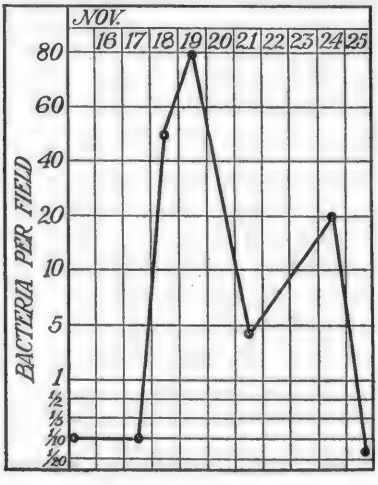
was examined bacteriologically every day. As soon as reinfection occurred the experiment was interrupted and the wound thoroughly sterilized. Every 4 days the surface of the wound was measured and the curve was plotted and compared with the calculated curve. The patients were kept in bed during the experiment and were under the supervision of a nurse day and night.

Influence of Distilled Water on the Rate of Healing of a Sterile Wound.

In the following experiments the wounds were flushed with distilled water from 2 to 4 hours every day.



Cicatrization curve.



Bacteriological curve.

TEXT-FIG. 1. Experiment 1. Case 646.

Experiment 1 (*Text-fig. 1*).—Case 646, age 25 years.

Nov. 17, 1916. Sterile wound in the calf, 12 sq. cm. in area. The wound is surgically aseptic. Flushing with distilled water for 2 hours. Then flushing with 30 per cent hypertonic solution for 2 hours. The dressing was kept in place until the following morning.

Nov. 18. The appearance of the wound has not changed. Surface area 12.1 sq. cm. 30 to 50 bacteria per field. Same dressing. Flushing with distilled water for 2 hours. Flushing with a hypertonic solution for 2 hours.

Nov. 19. The appearance of the wound has remained the same. Surface area 12.6 sq. cm. 50 to 100 bacteria per field. Dressing with chloramine paste, 10 parts per 1,000.

Nov. 20. Same dressing. Surface area 11.1 sq. cm.

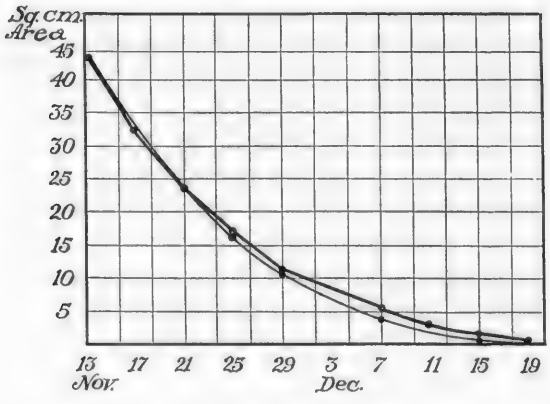
Nov. 21. Chloramine dressing, 4 parts per 1,000. Surface area 10.5 sq. cm. 4 bacteria per field.

Nov. 22. Flushing with distilled water for 4 hours. Chloramine dressing, 4 parts per 1,000 for 20 hours.

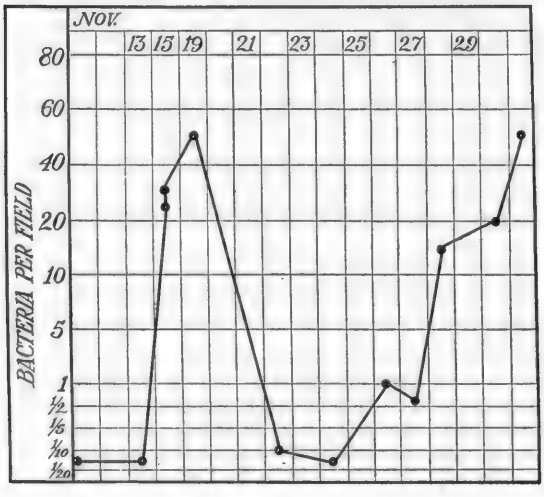
Nov. 23. Granulations smaller. Surface area 9.2 sq. cm. Flushing with distilled water for 4 hours. Chloramine dressing, 4 parts per 1,000.

Nov. 24. Same appearance. Surface area 8.4 sq. cm. 18 to 20 bacteria per field. Numerous cocci. Flushing with distilled water for 8 hours. Chloramine dressing, 10 parts per 1,000.

Nov. 25. 1 bacterium in 15 fields.



Cicatrization curve.



Bacteriological curve.

TEXT-FIG. 2. Experiment 2. Case 694.

Experiment 2 (*Text-fig. 2*).—Case 694, age 20 years. Sterile wound 33.5 sq. cm. in area.

Nov. 17, 1916. Flushing with distilled water for 2 hours, followed by 2 hours' flushing with 30 per cent hypertonic sodium chloride solution. The dressing was not renewed until the following morning.

Nov. 18. The wound appears the same. Surface area 30.4 sq. cm. 20 to 30 bacteria per field. Flushing with distilled water for 2 hours and hypertonic solution for 2 hours.

Nov. 19. Same appearance. Owing to the infection, the rate of cicatrization has diminished. The surface area is 29.6 sq. cm. 55 bacteria per field. Chloramine dressing, 10 parts per 1,000.

Nov. 20. Same appearance. The curve has caught up with the normal curve. Surface area 26 sq. cm. 20 bacteria per field. Chloramine paste, 10 parts per 1,000.

Nov. 21. Same appearance. Surface area 23.6 sq. cm. 4 bacteria per field. Chloramine dressing, 4 parts per 1,000.

Nov. 22. Chloramine dressing, 4 parts per 1,000. 1 bacterium per 8 or 10 fields.

Nov. 23. Same treatment. Surface area 17.3 sq. cm. The wound is sterile and the experiment with distilled water alone can be resumed.

Nov. 24. Flushing with distilled water for 8 hours. Dressing with chloramine paste, 4 parts per 1,000. 1 bacterium in 10 to 15 fields.

Nov. 25. Flushing with distilled water for 8 hours.

Nov. 26. Flushing with distilled water for 24 hours. In order to keep the wound sterile, an injection of Dakin's solution is made every 6 hours. Surface area 14.6 sq. cm.; calculated area 14 sq. cm. 1 bacterium per field.

Nov. 27. Flushing with distilled water for 24 hours. 1 bacterium in 3 fields.

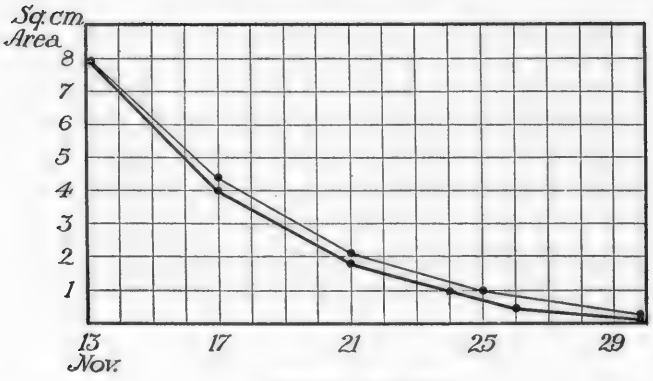
Nov. 28. Flushing with distilled water for 24 hours. Observed area 12 sq. cm. No apparent change. Calculated area 11.8 sq. cm. 10 to 15 bacteria per field.

Nov. 29. Flushing with distilled water for 24 hours.

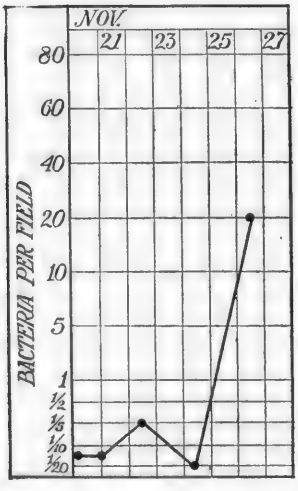
Nov. 30. Retardation of the healing process. Observed area 11 sq. cm.; calculated area 9 sq. cm. 15 to 20 bacteria per field. Flushing with distilled water for 24 hours.

Dec. 1. Experiment interrupted on account of infection. Application of compresses soaked in Dakin's solution; renewed six times in 24 hours.

Dec. 2. Same dressing. Innumerable small bacilli.



Cicatrization curve.



Bacteriological curve.

TEXT-FIG. 3. Experiment 3. Case 694.

Experiment 3 (*Text-fig. 3*).—Case 694, age 20 years.

Nov. 21, 1916. Sterile wound of the wrist, measuring 1.8 sq. cm. Since Nov. 13 the process of repair has been regular. Flushing with distilled water for 2 hours, followed as in the previous experiment by application of chloramine paste, 4 parts per 1,000, in order to prevent infection.

Nov. 22. Flushing with distilled water for 4 hours, followed by chloramine dressing. Observed surface 1.5 sq. cm.; calculated surface 1.8 sq. cm. 1 bacterium in 4 fields.

Nov. 23. Flushing with distilled water for 6 hours. Dressing with chloramine paste.

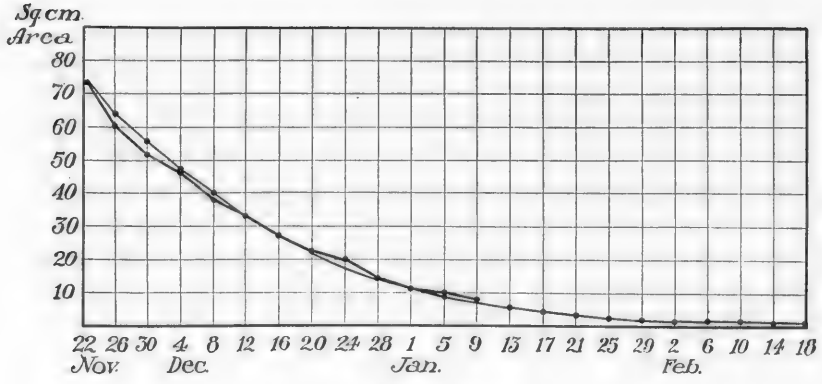
Nov. 24. Flushing with distilled water for 8 hours. Dressing with chloramine paste. 1 bacterium in 15 to 20 fields.

Nov. 25. Scale formed on the wound. Dry dressing.

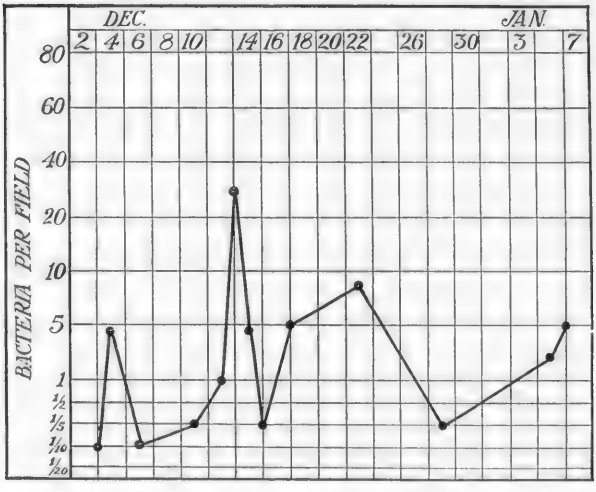
Nov. 26. Scale is removed. Area of the wound is 0.37 sq. cm.; calculated surface 0.7 sq. cm. 15 to 20 bacteria per field. Dressing with chloramine paste.

Influence of Hypertonic Sodium Chloride Solution on the Rate of Healing of a Sterile Wound.

In the following experiments the wounds were flushed with hypertonic sodium chloride solution.



Cicatrization curve.



Bacteriological curve.

TEXT-FIG. 4. Experiment 4. Case 639.

Experiment 4 (*Text-fig. 4*).—Case 639, age 36 years.

Dec. 1, 1916. The wound is practically sterile. 1 bacterium in 10 to 12 fields.

Dec. 2. Flushing with 40 per cent sodium chloride solution for 6 hours. Four injections with Dakin's solution. Surface area of wound 48.5 sq. cm.

Dec. 4. Flushing with 40 per cent sodium chloride solution for 12 hours. Four injections of Dakin's solution. Observed surface 46.5 sq. cm.; calculated surface 47.3 sq. cm. 3 bacteria per field.

Dec. 5. Same treatment.

Dec. 6. Same treatment. 1 bacterium in 8 to 10 fields.

Dec. 7. Same treatment.

Dec. 8. Same treatment. Six injections of Dakin's solution, instead of four. Area 38.5 sq. cm.; calculated area 39.7 sq. cm.

Dec. 9. Same treatment.

Dec. 10. Flushing with 40 per cent sodium chloride solution for 24 hours. Six injections of Dakin's solution. 1 bacterium in 8 fields.

Dec. 11. Same treatment.

Dec. 12. Same treatment. Surface of wound 33 sq. cm.; calculated surface 33 sq. cm. 1 bacterium per field. The curve as observed has finally overtaken the calculated curve and they now coincide exactly.

Dec. 13. Same treatment. 20 to 30 bacteria per field. The experiment is interrupted on account of infection.

Dec. 14. Sterilization of the wound by twelve flushings with Ringer solution.

Dec. 15. Same treatment. 1 bacterium in 6 fields.

Dec. 16. The experiment is resumed. A cake of agar-agar made of 40 per cent sodium chloride solution is applied to the wound during 12 hours. During the night the wound is sterilized by six flushings with Dakin's solution. Observed area 27 sq. cm.; calculated area 27.1 sq. cm. Sterile. The cake is applied for 9 hours.

Dec. 17. Same treatment. 4 bacteria per field.

Dec. 18. Same treatment. Surface observed 24.3 sq. cm.; calculated surface 24.2 sq. cm.

Dec. 19. A few bacteria.

Dec. 24. The wound is sterile. Agar cakes containing 50 per cent sodium chloride solution are applied during the day, and chloramine paste during the night.

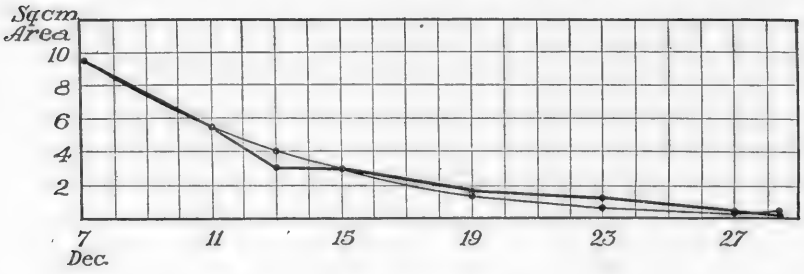
Dec. 28. The surface of the wound measures 14.2 sq. cm.; calculated surface 14.25 sq. cm. Same treatment.

Dec. 29. Same treatment.

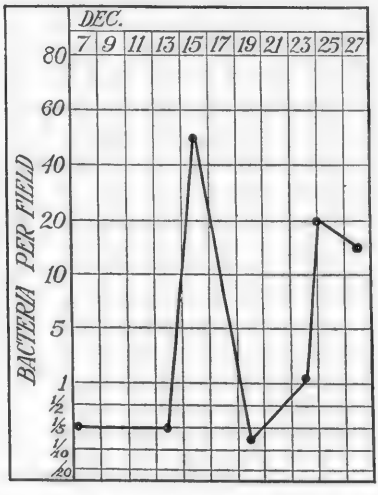
Dec. 30. Flushing with 80 per cent sodium chloride solution for 24 hours. Four flushings with Dakin's hypochlorite solution.

Jan. 1, 1917. Same treatment. Granulations congested. Small hemorrhages. Observed area 11 sq. cm.; calculated area 11.3 sq. cm.

Jan. 5. Same treatment with 80 per cent solution from Jan. 2 to 4. Observed area 10 sq. cm.; calculated area 9 sq. cm. 4 bacteria per field. The experiment is discontinued.



Cicatrization curve.



Bacteriological curve.

TEXT-FIG. 5. Experiment 5. Case 715.

Experiment 5 (*Text-fig. 5*).—Case 715, age 23 years; Arabian. Leg wound.

Dec. 7, 1916. Surface of wound 9.5 sq. cm. Dressing with Dakin's solution.

Dec. 11. Surface of wound 5.5 sq. cm.; calculated area 5.5 sq. cm. Flushing with 50 per cent sodium chloride solution for 24 hours, interrupted with two injections of Dakin's solution. Same treatment until Dec. 15.

Dec. 15. 30 to 50 bacteria per field. Four flushings with Dakin's solution in 24 hours. Surface observed 2.85 sq. cm.; calculated area 2.9 sq. cm.

Dec. 18. Flushing with hypertonic solution is substituted by the application of a cake of agar-agar containing 40 per cent sodium chloride. During the night three injections with Dakin's solution are made.

Dec. 19. Surface observed 1.5 sq. cm.; surface calculated 1.35 sq. cm. The injections are replaced by applications of chloramine paste, 10 parts per 1,000, the agar cakes being kept in contact with the wound for 12 hours every day.

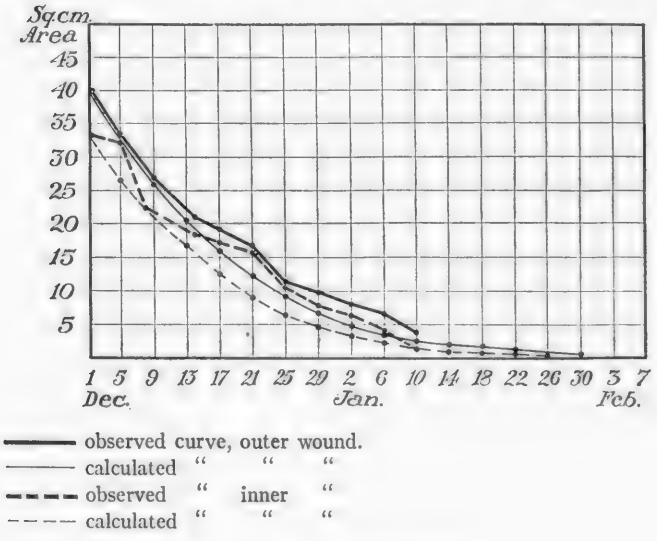
Dec. 23. Same treatment until Dec. 23. Observed area 1.1 sq. cm.; calculated area 0.6 sq. cm. The difference may be attributed to a slight infection. 2 bacteria per field.

Dec. 24. The 40 per cent agar cakes are replaced by a 50 per cent cake. The application of chloramine paste is continued during the night.

Dec. 27. Same treatment until Dec. 27, on which date it is calculated that healing will be effected. The wound, which is too small to be measured (less than 0.1 sq. cm.), is infected (10 to 15 bacteria per field), and this explains the retardation. Dressing with chloramine paste.

Dec. 29. The wound is completely healed.

Comparison of Distilled Water and of Hypertonic Sodium Chloride Solution on the Same Patient.



Cicatrization curve.

TEXT-FIG. 6. Experiment 6. Case 721.

Experiment 6 (*Text-fig. 6*).—Case 721. The patient has two wounds in the thigh, of about equal dimension, a small distance apart. The inner wound is flushed out with distilled water; the other with 40 per cent sodium chloride solution.

Dec. 3, 1916. Flushing of inner wound with distilled water for 12 hours. Four injections of Dakin's solution. Flushing of outer wound for 12 hours with 40 per cent sodium chloride. Four injections of Dakin's solution.

Dec. 4. 30 to 50 bacteria per field. Sterilization with Dakin's solution.

Dec. 8. Sterilization with Dakin's solution.

Dec. 12. Flushing for 24 hours per day under the same conditions described above, with six injections of Dakin's solution, continued until Dec. 16.

Dec. 17 and 18. Reinfection. Sterilization.

Dec. 20. Application to the external wound of an agar-agar cake, in the proportion of 40 per cent sodium chloride, and to the inner wound of a cake compounded with distilled water. 7 p.m. Dressing with chloramine paste. Same treatment until Dec. 27.

DISCUSSION AND SUMMARY.

In the study of the action of non-antiseptic substances on the rate of cicatrization, the chief obstacle encountered is the facility with which wounds become reinfected under an aseptic dressing. At the beginning of Experiment 1 the wound was sterile. It was subjected to flushing with distilled water for 2 hours, then to flushing with 30 per cent sodium chloride solution for another 2 hours. During that time no special precaution was taken to sterilize the wound and the dressing was left intact until the following morning. It was then found that the wound contained from 30 to 50 bacteria per field. The following day, after the wound had been subjected to the same treatment, the number of bacteria had increased to 50 and 100 per field, and as an immediate consequence the surface of the wound increased from 12 to 12.6 sq. cm. in 2 days. The wound was then dressed antiseptically and was found to be sterile 3 days later. Reinfection again took place the following day in spite of antiseptic dressing with chloramine paste 4 parts per 1,000, which was applied for 20 hours. In Experiment 2 similar results were observed. After 2 days of flushing with distilled water, the number of bacteria had increased to 50 per field. The wound was thereupon sterilized, but new reinfection ensued a few days later. Another wound on the same patient became reinfected under the same conditions after 1

day of sterile dressing. In none of the patients could the wounds be kept in a sterile condition throughout the whole experiment. It was impossible to maintain the sterility of a wound under aseptic dressing. Dakin's solution was therefore injected every 4 hours, or less often, according to the degree of infection, or chloramine paste was applied during the night. If there were 3 or 4 bacteria per field, the experiment was discontinued in order that the wound might be sterilized again. The cicatrization and bacteriological curves of Experiment 4 show that by the application of chloramine paste a wound may be maintained in an appropriately bacteriological condition for carrying out an experiment. Nevertheless, in spite of the antiseptic precautions taken, it was necessary to interrupt this experiment on two occasions, on December 13 to 15 and on December 18 to 22, in order that a complete sterilization of the wound might be effected. When the sterilization was performed as soon as the bacteria were discovered, little retardation occurred in the process of cicatrization. Moreover, the reinfection from the skin was often due to fine bacilli which have but mild retarding action on the rate of healing. The use of at least six flushings in 2 hours with Dakin's solution or of 12 hours' dressing with chloramine paste 10 parts per 1,000, was necessary to keep the wound in a condition of surgical asepsis.

The action of distilled water was studied in Experiments 1, 2, and 3. In Experiment 1 the wound was subjected to flushing with distilled water first for 2 hours, then 4 hours, and later for 8 hours per day. The wound was maintained in a condition of mild infection. No marked modification, either acceleration or retardation, was noted in the rate of repair during the period that the treatment was applied. From November 21 to 25 the wound was almost clean and the observed curve remained parallel to the calculated curve, showing that distilled water did not retard the rate of healing. In Experiment 2 the wound was subjected to uninterrupted flushing with distilled water, first for 2 and 8 hours, then for 24 hours. It was continued from November 24 to 30; *viz.*, for 112 hours out of 120, without the occurrence of any marked modification of the course of healing. The bacteriological curve showed that from November 22 to 27 inclusive the wound was kept aseptic. The slight retardation which occurred afterwards

was probably brought about by the infection. In Experiment 3 the wound was subjected to flushing with distilled water, first for 2, then for 4, 6, and 8 hours, a total of 20 hours in 4 days. From November 21 to 24 the wound remained surgically aseptic. No modification in the rate of healing occurred.

The action of the hypertonic sodium chloride solution was studied in a similar way. In Experiment 4 the wound was flushed at first with 40 per cent sodium chloride solution, from December 4 to 9 for 12 hours a day, and from December 10 to 13 for 24 hours a day, making a total of 144 hours out of 240 hours. At the end of this time the surface area of the wound coincided exactly with the calculated area. Owing to reinfection the experiment was suspended. From December 24 to 29 the wound was kept in contact with 50 per cent sodium chloride solution for 54 hours, and after December 30 flushing with 80 per cent solution for 24 hours a day was resorted to. The total amount of time involved in the above treatments was 174 hours with 40 per cent solution, 72 hours with 50 per cent solution, and 120 hours with 80 per cent solution. On January 1, the surface measured 11 sq. cm. and the calculated surface was 11.3 sq. cm. On January 5 the surface observed was 10 sq. cm. and the calculated surface was 9 sq. cm. It should be noticed that on January 5 the bacteria numbered 4 per field, which might account for the difference. In Experiment 5 the wound was flushed for 24 hours every day with 50 per cent sodium chloride solution from December 11 to 18, a total of 192 hours. From December 18 to 24 the wound was dressed with agar-agar cakes containing 40 per cent sodium chloride. The concentration was raised to 50 per cent from December 24 to 27. The cicatrization curve indicates only a slight retardation of the repair which can be attributed to infection when both cicatrization and infection curves are compared. The temporary acceleration on the 13th may have been due to the influence of the dressing, but as it did not occur again an experimental error is probably the cause of the change observed in the curve. In Experiment 6 two practically identical wounds at a distance of but a few centimeters from each other were located on the right thigh of Patient 721. The areas of the wounds were respectively 40 and 33 sq. cm. One of the wounds was flushed with distilled

water only. The other was subjected to the action of 40 per cent sodium chloride solution. From December 20 to 25 both wounds were in a condition of surgical asepsis. However, the cicatrization curves show that in spite of the difference of treatment the rate of healing was not modified.

The rate of healing of the wounds did not therefore apparently undergo any measurable modification under the influence of distilled water or hypertonic salt solution. It is well known that the osmotic changes of the medium have a marked influence on tissues deprived of circulation. But it seems that a tissue with normal circulation is protected by it against the changes of the osmotic pressure occurring at its surface. The above experiments show that apparently the conditions of the tissues of a wound are not modified by the changes of the osmotic pressure of the dressing. The beneficial effects of hypertonic sodium chloride solution on the sterilization of wounds and on the rate of healing recently described by various surgeons are possibly an illusion due to lack of precise technique.

CONCLUSIONS.

1. The flushing of an aseptic granulating wound with hypertonic sodium chloride solution or distilled water brings about an immediate reinfection.

2. Distilled water and hypertonic sodium chloride solution do not modify to a measurable extent the rate of healing of an aseptic wound.